UNIVERSIDADE DE SANTIAGO DE COMPOSTELA
Departamento de Química Analítica,
Nutrición e Bromatoloxía

Desarrollo de nuevos métodos de extracción en fase sólida para la preconcentración de metales traza en el agua de mar: evaluación de un soporte de C-18 y de polímeros de impronta iónica. Aplicación al estudio de la Ría de Arousa

TESIS DOCTORAL

Jacobo Otero Romaní

2009

Esta Tesis está dedicada a mis padres

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Memoria que para optar al grado de Doctor presenta:

Jacobo Otero Romaní

Santiago de Compostela, 2009.

UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

FACULTADE DE QUÍMICA

Dpto. de Química Analítica, Nutrición e Bromatoloxía

Dña. Pilar Bermejo Barrera y D. Antonio Moreda Piñeiro, Catedrática Numeraria y Profesor Titular del Dpto. de Química Analítica, Nutrición y Bromatología de la Facultad de Química de la Universidad de Santiago de Compostela,

INFORMAN,

Que la presente Tesis Doctoral, titulada "Desarrollo de nuevos métodos de extracción en fase sólida para la preconcentración de metales traza en el agua de mar: evaluación de un soporte de C-18 y de polímeros de impronta iónica. Aplicación al estudio de la Ría de Arousa" ha sido realizada bajo su dirección en el Dpto. de Química Analítica Nutrición y Bromatología de la Universidad de Santiago de Compostela. Y para que así conste firman la presente en Santiago de Compostela, a 30 de septiembre de 2009.

Pilar Bermejo Barrera

Antonio Moreda Piñeiro

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SIGLAS Y ABREVIATURAS

5-Br-PADAP	*2-(5-bromo-2-piridilazo)-5(dietilamino)fenol*
8-HQ	*8-Hidroxiquinolina*
AAS	*Espectrometría de Absorción Atómica*
AES	*Espectrometría de Emisión Atómica*
AFS	*Espectrometría de Fluorescencia Atómica*
AIBN	*2,2´- azo-bis-isobutironitrilo*
AMPC	*4-acetil-5-metil-1-fenil-1H-pirazola-3-acido carboxílico*
APD	*Bis(2-aminopiridil)dioxima*
APDC	*Pirrolidín ditiocarbamato amonico*
ARS	*Alizarín Rojo S*
ASV	*Voltamperometría de Redisolución Anódica*
C4RAHA	*Ácido calix[4]resorcinareno*
CAS	*Cromazurol S*
CCD	*Dispositivo de Acoplamiento de Carga*
CID	*Dispositivo de Inyección de Carga*
CPC	*Cloruro de cetil piridina*
CPE	*Extracción en Punto de Nube*
CTAB	*Bromuro de cetiltrimetilamonio*
CV-AAS	*Espectrometría de Absorción Atómica con Generación de Vapor Frío*
DBM	*Dibenzol metano*
DCP	*Plasma de Corriente Directa*
DDQ	*7-dodecenil-8-quinolinol*
DDTC	*Dietil-ditiocarbamato*
DDTP	*O,O dietil-ditiofosfato*
DEM	*2-(dietilamino) etil metacrilato*
DMG	*Dimetilglioxima*
DMSA	*Ácido dimercaptosuccínico*
DPASV	*Voltamperometría de Redisolución Anódica Diferencial de Pulsos*
DPC	*Difenilcarbazida*

DVB-VPD	*Divinilbenceno-vinilpirrolidona*
DZ	*Ditizona*
EDTA	*Ácido etilendiaminotetraacético*
EDXRF	*Fluorescencia de Rayos X de Energía Dispersiva*
EGDMA	*Etilen glicol-dimetacrilato*
ETAAS	*Espectroscopía de Absorción Atómica por Atomización Electrotérmica*
FAAS	*Espectroscopía de Absorción Atómica por Llama*
FIA	*Análisis por Inyección de Flujo*
GCB	*Negros de Carbón Grafitizados*
HDEHP	*Bis(2-etil-hexil) hidrogeno fosfato*
HMDC	*Hexametilen-ditiocarbamato*
HPLC	*Cromatografía Líquida de Alta Resolución*
ICP-MS	*Espectrometría de Masas por Plasma Acoplado Inductivamente*
ICP-OES	*Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente*
IIP	*Polímero de Impronta Iónica*
MAA	*Ácido metacrílico*
MC	*Colector Múltiple*
MIBK	*Metilisobutilcetona*
MIP	*Polímero de Impronta Molecular*
NaDDTC	*Dietilditiocarbamato sódico*
NIP	*Polímero de Impronta Blanco*
NN	*1-nitroso-2-naftol*
PAN	*1-(2-piridilazo)-2-naftol*
PDA	*Fotodiodos en Serie*
PDATA	*Ácido propilendiaminotetraacético*
PDT	*3-(2-piridil)-5,6-difenil-1,2,4-triacina*
PODPE	*(3-hidroxifosfinol-2-oxo-propil) acido fosfónico dibencil ester*
PS-DVB	*Poliestireno-divinilbenceno*
PSTH	*1,5-bis(2-piridil)-3-sulfofenil metileno tiocarbohidracida*
PTFE	*Politetrafluoroetileno*

PUF	*Espuma de Poliuretano*
RF	*Radiofrecuencias*
SEM	*Microscopía Electrónica de Barrido*
SPE	*Extracción en Fase Sólida*
TOPO	*Óxido de tri-n-octil fosfina*
TRIM	*Trimetil propano trimetacrilato*
TXRF	*Fluorescencia de Rayos X de Reflexión Total*
VHQ	*5-vinil-8-hidroxiquinolina*
VIS-UV	E*spectrofotometría Visible-Ultravioleta*
VP	*Vinilpiridina*
ZAN	*Zona Analítica Normal*
ZDP	*Zona de Precalentamiento*
ZRI	*Zona de Radiación Inicial*

RESUMEN

Durante la realización del presente trabajo de investigación se han desarrollado nuevos métodos de extracción en fase sólida para la preconcentración de metales traza en el agua de mar.

En primer lugar se ha desarrollado un método consistente en la precomplejación de los metales con 8–hidroxiquinolina (8-HQ), seguido de la adsorción de los complejos formados en un cartucho comercial de sílice C18 y la elución de los metales adsorbidos con un pequeño volumen de ácido nítrico 2M. Este método ha sido aplicado al estudio de la concentración de diversos metales traza (Al, Cu, Fe, Mn, Ni, Pb, Sn y Zn) en las aguas de la Ría de Arousa antes y después del accidente del petrolero "Prestige".

En segundo lugar se han sintetizado diversos polímeros de impronta iónica, siguiendo una novedosa metodología de síntesis polimérica denominada "polimerización por precipitación". Para ello, han sido empleados diferentes analitos plantilla (Ni^{2+}, Pb^{2+}), monómeros (4-vinilpiridina (4-VP), 2-dietilamino etil metacrilato (DEM)) y agentes complejantes (8-hidroxiquinolina (8-HQ), 5-vinil-8-hidroxiquinolina (5-VHQ)). Los polímeros obtenidos han sido caracterizados mediante distintas técnicas como la Fluorescencia de Rayos X de Energía Dispersiva (EDXRF), la Microscopía Electrónica de Barrido (SEM) y el Análisis Elemental, y han sido evaluados en base a diferentes estudios de adsorción / desorción de los distintos metales de interés (capacidad de carga, volumen de ruptura, exactitud, precisión…). Por último, los polímeros obtenidos han sido utilizados para la determinación de metales traza en muestras de agua de mar y materiales de referencia certificados mediante el empleo de diferentes técnicas analíticas como la Espectroscopía de Absorción Atómica por Atomización Electrotérmica (ETAAS), la Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente (ICP-OES) y la Espectrometría de Masas por Plasma Acoplado Inductivamente (ICP-MS).

INTRODUCCIÓN

1 – Determinación de metales traza en el agua de mar

Las aguas de mar se han mantenido inalteradas durante siglos, pero esta situación ha ido cambiando progresivamente debido a la actividad del hombre. Durante las últimas décadas se han producido multitud de asentamientos urbanos e industriales en costas y ríos, que traen como consecuencia un aumento progresivo de la contaminación de las aguas. Galicia es una comunidad especialmente sensible a esta realidad por tener una costa con numerosas Rías, en donde las aguas se encuentran particularmente estancadas siendo, como consecuencia, más sensibles a los contaminantes vertidos. Además de ello, las costas gallegas soportan un gran volumen de tráfico marítimo que constituye un factor de riesgo importante, como ha quedado constatado tras el hundimiento de grandes barcos que transportaban los más diversos materiales (petróleo, productos químicos...)

Las Rías de Galicia conforman una de las zonas pesqueras y marisqueras más importantes del mundo debido tanto a la riqueza de sus aguas, como a la existencia de criaderos de peces y moluscos. Es bien conocido que los moluscos filtran grandes cantidades de agua, por lo que son especialmente sensibles a la concentración de agentes contaminantes en sus organismos, como pueden ser los metales pesados. Si las aguas de mar se encuentran contaminadas, existe un gran riesgo de que estos metales, asimilados por los moluscos, pasen a la cadena alimentaria, con el consiguiente riesgo para la salud pública que ello supone. Por este motivo, se hace necesario disponer de una metodología analítica adecuada para la determinación de metales en el agua de mar.

1.1 – Composición del agua de mar

Los científicos han estudiado los océanos durante más de un siglo, pero todavía no han conseguido tener un conocimiento completo de su composición química [1]. Esto es debido, en parte, a la ausencia de métodos y procedimientos precisos para medir los constituyentes del agua de mar. Por el momento han sido identificados, al menos, 76

elementos químicos, la mayoría de ellos en cantidades extremadamente pequeñas. En general, los compuestos presentes en el agua de mar pueden dividirse en [2]:

1) Sólidos: materiales de tamaño superior a 0,45 μm

- Materia orgánica particulada

- Materia inorgánica particulada (minerales)

2) Gases disueltos en el agua de mar

- Conservativos: la cantidad de gas disuelta en un volumen determinado de agua permanece constante y solo es afectada por procesos físicos como la difusión o la interacción con otras masas de agua que contienen cantidades diferentes de gases disueltos (N_2, Ar, Xe, He…)

- No conservativos: gases que participan activamente en procesos químicos y biológicos y que, como consecuencia de ello, cambian constantemente su concentración (O_2, CO_2).

3) Coloides: materiales de tamaño inferior a 0,45 μm, pero que no están disueltos.

- Orgánicos

-Inorgánicos

4) Solutos: materiales disueltos de tamaño inferior a 0,45 μm.

- Solutos orgánicos

- Solutos inorgánicos

Atendiendo a su concentración, los componentes del agua de mar se pueden dividir en:

a) Componentes mayoritarios: aquellos cuya concentración está comprendida entre 0,05 y 750 mM.

b) Componentes minoritarios: aquellos cuya concentración se encuentra comprendida entre 0,05 y 50 μM.

c) Componentes traza: aquellos cuya concentración está comprendida entre 0,05 y 50 nM.

1.1.1 - Salinidad del agua de mar

Los oceanógrafos definen la salinidad del agua de mar como la cantidad total (medida en gramos) de sólidos disueltos en un kilogramo de agua. La salinidad de las aguas oceánicas varía de un sitio a otro. El agua de mar más salada (40‰) se encuentra en el Mar Rojo y en el Golfo Pérsico, donde la velocidad de evaporación del agua es muy elevada debido al calor. Las salinidades más bajas se encuentran en las aguas polares, donde la concentración de sal se diluye constantemente por fundición de los gigantescos icebergs [3].

La salinidad media de las aguas marítimas del planeta es del 35‰. Este dato fue obtenido por William Dittman en 1884, tras analizar 77 muestras de agua de mar recogidas en diversas partes del mundo durante la expedición científica del corvette británico H.M.S Challenger. La expedición del Challenger, establecida para estudiar la biología de las aguas marítimas, su composición química y sus propiedades físicas, comenzó en 1872 y finalizó 4 años después, tras recorrer 68.890 millas.

Las 77 muestras de Dittman son el primer conjunto de muestras de agua de mar de todo el mundo en el que se ha determinado la composición química de sus componentes mayoritarios. Datos recientes, obtenidos con técnicas analíticas y de muestreo más precisas, muestran ligeras diferencias con respecto a los resultados de Dittman, pero estos cambios no afectan a la validez global de su trabajo. El contenido de sal en mar abierto raramente es menor del 33‰ o mayor del 38‰ [3].

Existen más de 70 elementos disueltos en el agua de mar pero solo 6 de ellos constituyen más del 99% de todas las sales disueltas. El ión más abundante en el agua de mar es el cloruro (Cl^-), seguido del sodio (Na^+), el sulfato (SO_4^{2-}), el magnesio (Mg^{2+}), el calcio (Ca^{2+}) y el potasio (K^+). La Tabla 1 [4] muestra el tanto por ciento (en peso) de los iones mayoritarios presentes en el agua de mar.

Tabla 1. Compuestos iónicos mayoritarios presentes en el agua de mar.

IÓN	% (en peso)	IÓN	% (en peso)
Cl^-	55,03	Mg^{2+}	3,68
Na^+	30,59	Ca^{2+}	1,18
SO_4^{2-}	7,68	K^+	1,11

Las sales disueltas en el agua de mar proceden mayoritariamente de 3 grandes fuentes:

1- Erupciones volcánicas.

2- Reacciones químicas entre el agua de mar y la corteza oceánica.

3- La erosión de las rocas continentales.

1.1.2 - Concentración de metales traza

La concentración de metales traza en el agua de mar varía de un sitio a otro, encontrándose las diferencias más significativas entre las aguas oceánicas (mar abierto) y las aguas de estuarios y zonas costeras, que suelen estar más contaminadas y, por lo tanto, presentarán una mayor concentración de metales pesados. A priori, resulta difícil establecer un rango de concentraciones para cada elemento traza, sin embargo, con el objetivo de presentar unos niveles orientativos hemos recopilado algunos valores que se encuentran presentes en la bibliografía. La Tabla 2 muestra, según diferentes autores, los niveles de concentración de metales traza en el agua de mar abierto, así como la especie o especies químicas más probables bajo las que estos elementos se encuentran presentes.

1.2 – Importancia de la determinación de metales traza en el agua de mar

La protección y mejora del medio ambiente hace necesarias medidas concretas destinadas a proteger las aguas continentales y marinas contra la contaminación, para garantizar unas condiciones adecuadas para la vida acuática y los usos que de ellas se deriven.

Tabla 2. Concentración de metales traza en el agua de mar y especie o especies químicas más frecuentes bajo las que estos elementos se encuentran presentes.

ELEMENTO	ESPECIE PROBABLE	CONCENTRACION (µg/L)				
		Brown *et al.* [1]	Anthoni J. F. [5]	Merian E. [6]	Moreda J. [7]	Prego *et al.* [8]
Al	$Al(OH)_4$, $Al(OH)_3$	2,0	1,0	-	0,1-1,0	-
As	$HAsO_4^{2-}$	3,7	2,6	1,6	1,0-2,0	-
Cd	$CdCl_2$	0,1	0,1	0,1	0,0001-0,1	0,0001-0,1
Co	Co^{2+}, CoCO3	-	0,4	0,002	0,001-0,01	-
Cr	CrO_4^{2-}	0,3	0,2	0,2	0,1-0,3	-
Cu	Cu^{2+}, $CuCO_3$	0,1	0,9	0,2	0,03-0,4	0,03-0,3
Fe	$Fe(OH)_3$	2,0	3,4	0,1	0,01-0,1	0,001-0,06
Hg	$HgCl_4^{2-}$	0,03	0,1	0,002	0,0004-0,002	-
Mn	Mn^{2+}	0,2	0,4	0,01	0,01-0,2	-
Ni	Ni^{2+}, $NiCO_3$	1,7	6,6	0,6	0,1-0,7	0,1-0,7
Pb	Pb^{2+}, $PbCO_3$	0,0005	0,03	0,003	0,001-0,04	0,001-0,03
Sb	$Sb(OH)_6^-$	0,2	0,3	-	0,1	-
Se	SeO_4^{2-}, SeO_3^{2-}	0,2	0,9	0,1	0,04-0,2	-
Sn	$Sn(OH)_4$	0,01	0,8	0,001	0,0001-0,001	-
Sr	Sr^{2+}	8000	8100	-	7890	-
Ti	$Ti(OH)_4$	1,0	1,0	1,0	-	-
V	HVO_4^{2-}, $H_2VO_4^-$	2,5	1,9	1,9	1,0-2,0	-
Zn	Zn^{2+}, $ZnOH^+$	0,5	5,0	0,6	0,003-0,6	0,01-0,5

De esta forma, la Comunidad Económica Europea ha establecido una serie de directivas relativas a la calidad de las aguas continentales y marinas para diferentes usos, así como directivas relativas a la limitación de vertidos contaminantes a los ecosistemas acuáticos.

Las directivas comunitarias se centran fundamentalmente en los criterios de calidad del agua para usos recreativos [9] y para la cría de moluscos [10], limitándose en este ultimo criterio de calidad, los niveles guía e imperativos de diferentes contaminantes orgánicos y metales traza.

Las Rías de Galicia constituyen un espacio singular dentro de la geografía de nuestra Comunidad Autónoma. En ellas se encuentra una gran riqueza natural de fauna y paisajística, y de importante valor económico a la vez, que viene conviviendo con asentamientos urbanos y actividades industriales de cierta entidad. El deterioro que a través de los años estas actividades, u otras de nueva implantación, pueden causar por efectos puntuales o acumulativos a la calidad de sus aguas, hace necesario que se utilicen todos los instrumentos a nuestro alcance para prevenir y corregir estas consecuencias perniciosas.

La Ley de Protección de la Calidad de las Aguas de las Rías de Galicia, publicada en el Boletín Oficial del Estado el Martes 25 de Septiembre de 2001 [11], pretende la protección de la calidad de las aguas de las Rías de Galicia mediante el establecimiento de objetivos de calidad, así como de límites para la emisión de vertidos de aguas residuales a las Rías gallegas. Entre los objetivos de calidad, la normativa establece que se han de cumplir parámetros bacteriológicos, físicos y químicos. Entre estos parámetros químicos se establece como objetivo de calidad que la concentración de ciertos metales en las aguas de las rías gallegas no superen los límites especificados en la Tabla 3 [11]. Del mismo modo, la normativa establece la concentración puntual, diaria y mensual máxima de metales que pueden contener las aguas residuales vertidas a las rías gallegas por las industrias (Tabla 4) [11].

Con el objetivo de verificar el cumplimiento de la normativa comunitaria vigente, se hace necesario el desarrollo de métodos analíticos que nos permitan determinar con fiabilidad la concentración del mayor número posible de metales traza presentes en el agua de mar. Sin embargo, existen dos problemas fundamentales para el análisis de los metales en este medio: la composición salina del agua de mar y la baja concentración de los metales en la misma.

Tabla 3. Límites de concentración especificados como objetivo de calidad en las aguas de las rías gallegas (BOE núm. 230, Pág. 35528, 2001).

METAL	CONCENTRACION (µg/L)
As total	50
Cd	5
Cr total disuelto	50
Cr (VI)	5
Cu disuelto	No figura
Hg	0,5
Ni disuelto	No figura
Pb disuelto	50
Se disuelto	1
Zn total	No figura

Tabla 4. Límites de concentración de metales presentes en las aguas residuales vertidas a las Rías de Galicia (BOE núm. 230, Pág. 35528, 2001).

METAL	MEDIA MENSUAL (mg/L)	MEDIA DIARIA (mg/L)	VALOR PUNTUAL (mg/L)
As	1	3	5
Cd	0,2	0,4	0,4
Cr total	0,5	2	4
Cr (VI)	0,2	0,4	0,5
Cu	0,5	2,5	4
Hg	0,05	0,1	0,1
Ni	3	6	10
Pb	0,5	1	2
Se	0,05	0,1	0,2
Sn	10	15	20
Ti	1	3	5
Zn	3	6	10

1.3 – Dificultades del análisis de metales traza en el agua de mar

Las aguas con un alto contenido en sales disueltas son medios en los que los métodos clásicos de análisis para determinar la concentración de metales traza son difíciles de aplicar. Las altas concentraciones de metales alcalinos (Na^+, K^+), alcalinotérreos (Mg^{2+}, Ca^{2+}) y halogenuros (Cl^-) pueden ser fuentes de interferencias físicas y químicas que afecten a la precisión de las medidas, así como al funcionamiento de los instrumentos analíticos empleados para realizar las mediciones [12].

La concentración extremadamente alta de metales alcalinos, alcalinotérreos y halogenuros en el agua de mar, junto con los niveles extremadamente bajos de los metales traza, hace difícil o imposible la determinación directa de estos metales por la mayoría de los instrumentos analíticos convencionales [13]. Por este motivo, han sido desarrollados una gran variedad de métodos de preconcentración / separación con el fin de aumentar la concentración de los metales traza, y separarlos de la matriz salina en la que se encuentran presentes.

1.4 - Preconcentración de metales traza en el agua de mar

Un método ideal de preconcentración de metales traza en el agua de mar debe poseer las siguientes características:

- Debe permitir simultáneamente el aislamiento del analito de la matriz y proporcionar un factor de preconcentración adecuado.
- Debe ser un proceso simple que requiera la introducción de pocos reactivos, con el fin de minimizar la contaminación y, de este modo, producir un blanco de muestra menor, reduciendo así los límites de detección.
- Debe producir una disolución final que sea comparable con las disoluciones empleadas para realizar el calibrado.

Entre los métodos más ampliamente utilizados para la preconcentración de metales traza en el agua de mar se encuentran los procesos de extracción líquido-líquido [14], basados en la complejación selectiva del analito con un agente quelatante y su posterior extracción en un pequeño volumen de disolvente orgánico; los procesos de coprecipitación [15-20], basados en la precipitación selectiva del analito mediante la adición de un agente precipitante; y los métodos de extracción en fase sólida. En las Tablas 5 y 6 se muestra un resumen de los métodos desarrollados durante los últimos 5 años (2004-2009) para la preconcentración de metales traza en el agua de mar. Si se desea más información sobre el tema se puede consultar la revisión publicada por Prasada-Rao *et al.* [21], en la que se resumen los métodos desarrollados desde 1995 hasta 2005 y la publicada por Nickson *et al.* [22] en la que se resumen los métodos de extracción en fase sólida para la preconcentración de metales traza en aguas naturales desarrollados hasta 1995. Otra revisión interesante es la publicada por Hirose [23] en el año 2006, que trata sobre la especiación química de metales traza en el agua de mar.

2 – Extracción en fase sólida de metales traza

2.1 - Principios de la extracción en fase sólida

La extracción en fase sólida (Solid-Phase Extraction, SPE) es una técnica de extracción basada en la partición selectiva de uno o más compuestos entre dos fases, una de las cuales es un sólido adsorbente [24]. La segunda fase suele ser un líquido, pero también puede ser una emulsión, un gas o un fluido supercrítico.

El objetivo principal de la extracción en fase sólida es el aislamiento selectivo de los analitos de una muestra y la eliminación de la matriz interferente. Los compuestos de interés pueden ser adsorbidos por el sólido, o bien permanecer en la otra fase, mientras que en la fase sólida quedan retenidos los compuestos interferentes.

Tabla 5. Resumen de los procedimientos "off-line" para la preconcentración de metales traza en agua de mar desarrollados durante los años 2004-2009.

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Co, Cu, Fe, Mn, Ni	Extracción en fase sólida (SPE) empleando una resina quelatante iminodiacetica	ICP-OES	6, 8, 6, 5 y 6 ng L^{-1} para Co, Cu, Fe, Mn y Ni resp.		[25]
Cd, Cu, Ni, Pb	Complejación de los metales con dietilditiocarbamato sódico (NaDDTC) y extracción en punto de nube (CPE) empleando un surfactante no iónico (Tritón X-114)	ICP-OES	0,030; 2,1; 0,62 y 0,27 µg L^{-1} para Cd, Pb, Cu y Ni, resp.	Desde 3,1% hasta 6,6% (n = 10)	[26]
Cu	Complejación del Cu(II) con cromazurol S (CAS) y bromuro de cetiltrimetilamonio (CTAB), y adsorción de los complejos en una mini columna empaquetada con algodón.	ICP-OES	40 ng L^{-1}	1,3%	[27]
Cd, Co, Cu, Mn, Mo, Ni, Pb, U, V, Zn	Preconcentración empleando un disco quelatante	ICP-MS			[28]
Cd, Cu, Pb, Ni	Adsorción en una columna de clinoptilolita impregnada con 1-(2-piridilazo)-2-naftol (PAN)	FAAS	0,28; 0,12; 0,44; y 0,46 µg L^{-1} para Cd, Cu, Pb y Ni resp.	Desde 1,2% hasta 1,4% (n = 10)	[29]
Hg	Preconcentración "en baño" empleando un polímero de impronta iónico.	CV-AAS	0,006 µg/L	Desde 5% hasta 9%	[30]
Sb (III) y Sb total	Microextracción en gota con generación "in-situ" de estibilina	ETAAS	25 pg mL^{-1}	4,7%	[31]
Mo	Preconcentración en una resina quelatante de TSK-8HQ	MC-ICP-MS			[32]
Cr(III) y Cr(VI)	Extracción selectiva y preconcentración mediante alúmina modificada con 2-piridina carboxialdehido tiosemicarbazona				[33]
Be	Extracción en punto de nube (CPE) empleando micelas mixtas del surfactante catiónico cloruro de cetil piridina (CPC) y del surfactante no iónico Triton-X-114	SPD	0,2 ng mL^{-1}	0,16 % (n=5)	[34]
Cr(III) y Cr(VI)	Extracción selectiva y preconcentración mediante alúmina modificada con isatin-tiosemicarbazona				[35]
Cr(III) y Cr(VI)	Extracción en fase sólida con alúmina modificada con tiosemicarbacida				[36]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn	Preconcentración empleando una resina quelatante de acido etilendiaminotriacetico	ICP-MS		<9%	[37]
Fe(II) y Fe(III)	Extracción líquido-líquido con metilisobutilcetona (MIBK) empleando 4-acetil-5-metil-1-fenil-1H-pirazola-3-acido carboxílico (AMPC) como agente complejante para el Fe(III)	FAAS	0,24 μg L^{-1} para Fe(III)	<2,1% (n = 8)	[38]
Cd, Cu, Pb	Sensores libres de Hg mediante una combinación de monocapas de tiol auto-ensambladas sobre soportes mesoporosos y electrodos de Nafión sobre vidrio grafitizado	SWV	0,5 y 2,5 μg L^{-1} para Pb y Cd resp.	5% (n=8)	[39]
Hf, U, Th, Zr	Extracción en punto de nube mediante complejación de los metales con dibenzolmetano (DBM) empleando Triton X-114 (0.2%, w/v) como surfactante.	ICP-OES	Desde 0,1 hasta 1,0 μg L^{-1}	<6,1% (n=7)	[40]
Cd	Adsorción en capas finas activadas con el extractante Aliquat 336 preparado por impregnación directa de soportes poliméricos o mediante inclusión física en una matriz de triacetato de celulosa	EDXRF			[41]
Cu, Cd, Co, Pb, Ni, Zn	Extracción en fase sólida de los quelatos metálicos formados con pirrolidín ditiocarbamato amónico (APDC) sobre nanotubos de carbono	FAAS	Desde 0,30 hasta 0.60 μg L^{-1}		[42]
Cd, Cu, Hg, Pb	SPE sobre sílica cubierta con nanopartículas magnéticas modificadas con γ -mercapto propil trimetoxi silano	ICP-MS	≥24, 92, 107 y 56 pg L^{-1} para Cd, Cu, Hg y Pb, resp.	Desde 3,7% hasta 9,6% (n = 7)	[43]
Cr, Cu, Pb	SPE empleando una resina quelatante fluorada de estireno-divinilbenceno modificada con β -dicetona	ICP-OES		<5%	[44]
Cd, Co, Cr, Mn, Ni, Pb	Preconcentración sobre Chromosorb 101 modificado con bacillus thuringiensis var. israelensis	FAAS	Desde 0,37 hasta 2,85 μg L^{-1}		[45]
Cd, Co, Ni, Pb	Preconcentración sobre microesferas de poliestireno-divinilbenceno modificadas con 8-hidroxiquinolina	TXRF			[46]
Cu y Ni	SPE con sílica gel modificada con tetraamina de trietileno	FAAS	0,22 y 0,36 μg L^{-1} para Ni y Cu resp.		[47]
Metales traza	SPE empleando una minicolumna empaquetada con una resina quelatante	ICP-MS			[48]
Cd, Cu, Fe, Ni, Pb, Zn	SPE empleando una resina quelatante de poliamino-ácido policarboxílico (NOBIAS Chelate-PA1)	ICP-OES / MIP-MS			[49]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Ce, Gd, La, Nd, Pr, Sm	Extracción en fase sólida empleando Poli(β -esteril)-(1,2-metanofulereno-C60)-61- acido formohidroxámico	ICP-MS			[50]
Th y U	Separación y determinación de Th(IV) y U(VI) empleando arsenazo III como reactivo colector mediante flotación-método espectrofotométrico	SPD	$1,7 \times 10^{-7}$ mol dm^{-3}	Desde 1,7% hasta 1,87% (n = 7)	[51]
Co, Cu, Ni	Extracción en fase sólida empleando C activado modificado con ditioxamida (acido rubeanico)	FAAS	0,50 μg/L para Cu; 0,75 μg/L para Ni y 0,80 μg/L para Co	<2% (n =3).	[52]
Hg	Extracción en fase sólida empleando sílica gel modificada con naranja de xilenol	VIS-UV			[53]
Fe, Mn, Pb, Zn	Complejación de los iones con dietilditiocarbamato y preconcentración con Chromosorb 105	AAS		<6%	[54]
As, Cd, Co, Cu Cr, Fe, Mn, Mo, Ni, Pb, V, U, Zn	Extracción líquido-líquido empleando metil-isobutil cetona (MIBK) y ditiocarbamato como agente complejante	ICP-MS			[55]
Co, Cu, Ni	Preconcentración empleando una resina de polivinil cloruro – polietileno poliamina	FAAS	0,81; 0,98 y 0,57 μg/L para Cu, Ni y Co, resp.	Desde 2,3% hasta 5,0% (n=7)	[56]
Cd, Co, Cu, Mn, Mo, Ni, Pb, U , V, Zn	Preconcentración con una minicolumna quelatante	ICP-MS			[57]
Cd , Co, Cu, Mn, Ni, Pb	Coprecipitación con hidróxido de Dy(III)	AAS	Desde 14,1 hasta 25,3 μg/L.	<10%	[58]
Pb	Preconcentración empleando naftaleno microcristalino modificado con ácido esteárico	FI-FAAS		Desde 1,2% hasta 5,1%	[59]
Metil-mercurio	Extracción en fase sólida empleando un polímero de impronta iónico	ICP-OES / HPLC-DAD			[60]
Hf, Nb, Ta, W, Zr	Extracción en fase sólida empleando una resina de TSK-8-hidroxiquinolina	ICP-MS	Desde 0,009 hasta 0,15 pmol/kg	Desde 2% hasta 4%	[61]
Ce	Extracción líquido-líquido con un derivado del ácido calix[4]resorcinareno (C4RAHA)	ICP-OES			[62]
Hg	Adsorción y separación empleando sílica gel modificada con 2-aminotiazol	DPASV	0,10 μg L^{-1}	Desde 3,5% hasta 4,1% (n = 6)	[63]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Cd, Co, Cu, Cr, Fe, Mn, Pb	Complejación con oxina alfabenzoina seguido de la adsorción en una resina Diaion SP-850	FAAS	0,65; 0,42; 0,28; 0,73; 0,30; 0,47 y 0,50 μg L^{-1} para Cr, Mn, Fe, Co, Cu, Cd y Pb resp.	<8%	[64]
Cd, Cu, Ni, Pb, Zn	1) Extracción líquido-líquido con APDC/DDDC-Freon TF 2) Extracción en fase sólida con Amberlita XAD-4 y Dowex Optipore V-493 3) Coprecipitación con Cobalto.				[65]
Cd, Co, Cr, Cu, Ni, Pb	Preconcentración con arcilla Celtek	FAAS	0,25; 0,32; 0,73; 0,45; 0,50 y 0,41 μg/L para Cu, Cd, Pb, Cr, Ni y Co resp.	<10%	[66]
Elementos de las Tierras Raras (REEs)	Preconcentración empleando una minicolumna empaquetada con una resina quelatante	ICP-MS	Desde 0,06 hasta 0,5 ng/L		[67]
Cu	Preconcentración empleando un adsorbente quelatante (esferas de agarosa activadas con bis(2-aminopiridil)dioxima (APD) enlazado químicamente)	FAAS	0,16 μg L^{-1}		[68]
Cd, Co, Cu, Ni, Pb, Zn	SPE empleando cartuchos de C18 (Sep-Pak) modificados con 8-HQ				[69]
Eu, Lu, Nd	SPE empleando naftaleno modificado con 5,7-dicloro-8-hidroxiquinolina	FAAS	40; 4,0 y 30 μg L-1 para Nd, Eu y Lu resp.	Desde 1,8% hasta 2,5%.	[70]
Elementos de las Tierras Raras	Preconcentración empleando un tubo poroso de PTFE modificado con bis(2-Etil-Hexil) Hidrogeno Fosfato (HDEHP)	ICP-MS		Desde 1,1% hasta 16% (n = 3)	[71]
U y Th	Preconcentración en un polímero selectivo de iones multidentado (AXAD-16-MOPPA)			3,5%	[72]
Lantánidos y actínidos	Preconcentración en un polímero selectivo de iones multidentado (AXAD-16-TDHCHPA)			4,1% (n=3)	[73]
Co, Cu, Zn	Preconcentración empleando una resina de intercambio aniónico Dowex modificada con 4-(2-piridilazo)-resorcinol	EDXRF	1,53; 0,31 y 0,21 μg L^{-1} para Co, Cu y Zn resp.	Desde 2,1% hasta 3,4% (n=5)	[74]
Ni, Cd, Co, Mg	Preconcentración empleando cadenas cortas de péptidos de acido aspártico y cisteína inmobilizadas	FAAS			[75]
Metales traza	Preconcentración en “tándem” con Chelex-100	ICP-MS			[76]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Hg	Complejación con N,N'-bis(2-mercaptofenil) etanodiamida y extracción en fase sólida empleando una columna de sílica gel	SPD	0,34 y 0,015 μg mL^{-1} cuando los factores de preconcentración son 2 y 50, resp.	1,6% (n=9)	[77]
Cd, Co, Cu, Mn, Ni, Pb, U, Y, Zn, y Elementos de la Tierras Raras (REEs)	Preconcentración empleando una minicolumna empaquetada con una resina quelatante	ICP-MS			[78]
Elementos de las Tierras Raras	Preconcentración en una microcolumna empaquetada con nanotubos de carbono	ICP-AES	Desde 3 hasta 57 ng L^{-1}	<6%	[79]
Metales traza	Preconcentración con quitosano etilen diamino-N,N,N'-triacetato	ICP-MS			[80]
Cd, Cu, Mn, Ni, Pb, U, Zn	Fotólisis UV y adsorción en una resina quelatante	ICP-MS	Entre 0,6 ng L^{-1} para el Cd y 33 ng L^{-1} para el Ni		[81]
Ni	Sistema de membranas líquidas	AAS	0,012 μg L^{-1}	4,7%	[82]
Cd, Cu, Ni	Sistema de membranas líquidas	FAAS			[83]
Al, Cd, Cu, Mn, Ni, Pb, Zn	Sistema de membranas líquidas	ICP-MS			[84]
Ag, Cd, Cu, Pb	Sensores electroquímicos con nanopartículas magnéticas modificadas con DMSA	ICP-MS	<1 ppb		[85]
Ag, Co, Cu, Cr, Fe, Mn, Ni, Pb	Extracción en punto de nube con un surfactante no iónico (Triton X-114) y APDC como agente quelatante	ETAAS	0,003; 0,008; 0,003; 0,006; 0,015; 0,002; 0,009 y 0,01 ng mL^{-1} para Ag, Co, Cr, Cu, Fe, Mn, Ni y Pb resp.	Desde 11% hasta 25%	[86]
Cd, Cu, Mn, Ni, Zn	Gradientes difusivos en capas finas preparados con APA-gel como capa difusiva y resina Chelex-100 como agente vinculante	ICP-MS			[87]
Hg	Preconcentración en un electrodo modificado químicamente con 3-(2-tioimidazol) propil sílica gel	PASV cíclico y diferencial	5 μg L^{-1}	Desde 2,5% hasta 3,0% (n=6)	[88]
Hg	Adsorción selectiva sobre un electrodo de pasta de carbono modificado con arcilla		0,1 μg L^{-1}		[89]
La, U, Th	Extracción empleando una resina de Amberlita XAD-16 modificada químicamente con (3-hidroxifosfinol-2-oxo-propil) acido fosfónico dibencil ester (POPDE)	VIS-UV/ FAAS	Entre 10 y 20 ng cm^{-3}	5,2%	[90]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Cd, Cu, Fe, Ni, Pb, Tl, Zn	Separación mediante flotación empleando Co(III) hexametilen ditiocarbamato	FAAS / ETAAS	0,010; 0,034; 0,305; 0,290 y 0,032 µg/L para Cd, Cu, Ni, Pb y Tl resp. (Flotación/ETAAS) 0,890 y 0,994 mg/L para Fe y Zn resp. (Flotación/FAAS)		[91]
Cu, Mn, Zn	Preconcentración empleando Amberlita XAD-7 y Dowex 1X-8 cargadas con Alizarín Rojo S (ARS)	FAAS		Desde 0,2% hasta 0,4% para la XAD 7-ARS y desde 0,3% hasta 0,5% para la Dowex-ARS	[92]
U	Extracción en fase sólida empleando XAD-4 modificado con dibenzol metano (DBM), 8-hidroxiquinolina y oxido de tri-n-octil fosfina (TOPO)	SPD			[93]
Mn	Complejación de los analitos con 4-(2-piridilazo-resorcinol) y extracción en fase sólida de los quelatos formados con carbón activado	FAAS	16 ng/L		[94]
Co y Ni	Preconcentración en un polímero de polivinil pirrolidona (PVP)	GFAAS			[95]
Cd, Pb, Th, U	Preconcentración empleando Amberlita XAD-16 modificada químicamente con oxiacetona acetamida			<4,1% (n=3)	[96]

Tabla 6. Resumen de los procedimientos "on-line" para la preconcentración de metales traza en agua de mar desarrollados durante los años 2004-2009.

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Elementos de las Tierras Raras	Preconcentración empleando una columna quelatante empaquetada en una jeringuilla	ICP-MS	Desde 0,005 pg mL^{-1} hasta 0,09 pg mL^{-1}		[97]
Cu	Extracción con fibra de seda	ETAAS	8,0 ng/L	2,2%	[98]
Cd, Co, Cr, Cu, Fe, Mn	Extracción en punto de nube (CPE) mediante complejación de los metales con 1-(2-tenoil)-3,3,3-trifluoroacetona a pH 6.0 en presencia de un surfactante no iónico (Triton X-114)	ICP-OES	Desde 0,1 hasta 2,2 µg/L	<4,6%	[99]
Ag, Bi, Cu, Ga, In, Mo, Ni, U, V	Preconcentración empleando una minicolumna de quitosano modificado con 3,4-dihidroxi acido benzoico	ICP-OES	Ag 0,08; Bi 0,9; Cu 0,07; Ga 0,9; In 0,9; Mo 0,08; Ni 0,09; U 0,9; y V 0,08 ng/mL		[100]
Elementos de las Tierras Raras	Preconcentración con una resina de ácido alquil fosfínico	ICP-MS	Desde 1,43 pg/L (Ho) hasta 12,7 pg/L (La)	<5% (n = 6)	[101]
Pd	Preconcentración empleando sílica gel modificada con 1,5-bis(di-2-piridil)metileno tiocarbohidracida (DPTH-gel) y con una resina de intercambio iónico (Dowex 1X8-200) modificada con [1,5-Bis(2-piridil)-3-sulfofenil metileno tiocarbohidracida (PSTH)				[102]
Pt	Preconcentración empleando una minicolumna empaquetada con una resina de intercambio aniónico (Dowex 1 X 8-200) modificada con 1,5-bis(2-piridil)-3-sulfofenil metileno tiocarbohidracida (PSTH)	GFAAS / ICP-OES	1 ng mL^{-1} (FI-GF-AAS) 7,4 ng mL^{-1} (FI-ICP-AES)	1,6% (FI-GF-AAS) y 3,06% (FI-ICP-AES)	[103]
Ag, Au, Cd, Cu, In, Ni, Pb, Rh, Tl, Zn	Complejación y preconcentración en una microcolumna adsorbente	ICP-MS	Desde 3 hasta 20 ng/L		[104]
Cd	Adsorción en una minicolumna empaquetada con Chelex-100	FAAS	17 ng L^{-1}		[105]
Cd, Cu, Pb, Zn	Preconcentración empleando una minicolumna empaquetada con fibras de algodón tiol	ICP-MS		< 7%	[106]
Hg	Complejación con bromuro de tetrabutilamonio y preconcentración en una minicolumna empaquetada con ODS de 5 µm	HPLC-detector fotométrico	0,5 µg L^{-1}		[107]

Elemento/s	Procedimiento	Detección	Límite/s de Detección	Coef. de Variación	Ref.
Cd y Pb	Complejación de los metales con 8-HQ seguido de la adsorción de los complejos en una columna cromatográfica empaquetada con Amberlita XAD-2	GFAAS		10%	[108]
Cd	Electrodos de bismuto recubiertos de nafión y electrodos de mercurio recubiertos de nafión	ASV-ICP-MS			[109]
Fe (II) y Fe(III)	Preconcentración en las paredes internas de un reactor tubular de nylon	FIA-FAAS	1,8 $\mu g\ L^{-1}$		[110]
Cd	Preconcentración empleando una resina quelatante de quitosano modificado con 8-HQ	FI-FAAS	0,1 $\mu g\ L^{-1}$ para Cd(II) y 0,4 $\mu g\ L^{-1}$ para Cu(II)	Desde 0,7% hasta 1,5%	[111]

Cuando la SPE se lleva a cabo en una única etapa de equilibrio (en baño), el proceso es similar a la extracción líquido – líquido, con la diferencia de que el adsorbente sólido reemplaza al disolvente inmiscible [112]. Una vez alcanzado el equilibrio, las dos fases son separadas físicamente mediante decantación, filtración, centrifugación, o un proceso similar. Si los analitos quedan retenidos en la fase sólida, pueden ser desorbidos mediante el lavado con un disolvente selectivo adecuado. Si permanecen en la fase líquida, pueden ser recuperados mediante evaporación del disolvente, separación cromatográfica o recristalización. Este proceso no es muy eficiente y puede conducir a recuperaciones incompletas de los analitos. Los beneficios reales de la SPE se logran cuando el adsorbente se encuentra eficientemente empaquetado en una columna, formando un lecho uniforme que permita una distribución de flujo adecuado. La SPE en columna se encuentra regida por los mismos principios que la Cromatografía Líquida. En cada columna se pueden generar de 10 a 50 "platos cromatográficos" o, lo que es lo mismo, de 10 a 50 etapas de equilibrio, lo que nos permite obtener recuperaciones muy superiores a las que se obtiene mediante una sola etapa de extracción [113].

La separación efectiva mediante SPE depende primordialmente de la elección del sólido adsorbente y de los disolventes selectivos empleados para la elución. Después de considerar la naturaleza química y la polaridad de los componentes de la muestra, y del disolvente en el que se encuentran, se elige un adsorbente sólido, de actividad y tamaño de partícula adecuado, y se empaqueta en una columna, fabricada con un material inerte. El lecho cromatográfico debe ser homogéneo y estar libre de huecos y canales, para asegurar la eficiencia del contacto con la muestra. Si fuera necesario, se puede emplear un disolvente adecuado para equilibrar y limpiar el lecho cromatográfico. A continuación, la muestra se introduce por gravedad, presión positiva o vacío, a una velocidad de flujo controlada. Diversos disolventes, o mezclas de disolventes, son usados a continuación para desorber selectivamente los compuestos deseados en una secuencia adecuada.

Las dificultades en la SPE en columna surgen normalmente debido a la elección inadecuada del adsorbente, al pobre empaquetamiento del adsorbente en la columna, a la elección inadecuada de los disolventes para realizar la elución, y a la utilización de velocidades de flujo excesivas, que no permiten alcanzar el equilibrio.

2.1.1 - Interacciones

Las interacciones entre el sólido adsorbente y los analitos de una muestra pueden ser de tres tipos [114]:

- **Interacciones apolares**

Las interacciones apolares se producen entre los residuos hidrocarbonados de los grupos funcionales del sólido adsorbente y los analitos de la muestra. Teniendo en cuenta que la mayoría de los compuestos orgánicos posee una estructura apolar, estos pueden ser fácilmente adsorbidos mediante fuerzas de Van der Waals. La excepción la constituyen aquellos compuestos que poseen un gran número de grupos polares o iónicos, que pueden enmascarar el carácter apolar del esqueleto carbonado.

Adsorbentes típicos con un pronunciado carácter apolar son las sílices modificadas C18 y C8. Estas resinas muestran, no obstante, una baja selectividad, debido a que sus grupos funcionales, los sustituyentes alquilo, pueden interaccionar con casi todos los analitos apolares. En general, los compuestos apolares presentes en una disolución polar son fácilmente adsorbidos en adsorbentes apolares y eluidos con disolventes apolares.

- **Interacciones polares**

Las interacciones polares incluyen la formación de puentes de hidrógeno, interacciones dipolo – dipolo e interacciones Π - Π que pueden ocurrir entre el sólido adsorbente y los grupos funcionales de los analitos. Algunas de esas interacciones son posibles entre grupos amino, hidroxilo y carbonilo, al igual que anillos aromáticos, dobles enlaces y grupos con heteroátomos como el nitrógeno, el azufre, el fósforo y el oxígeno.

Adsorbentes típicos para interacciones polares son la sílice no modificada, o las sílices modificadas con grupos -CN, $-NH_2$ y -OH. En general, los compuestos polares presentes en una disolución apolar son fácilmente adsorbidos en adsorbentes polares y eluidos con disolventes polares.

- **Interacciones iónicas**

Las interacciones iónicas se producen entre un analito con carga positiva o negativa, y un sólido adsorbente con grupos funcionales de la carga opuesta. La retención mediante interacciones iónicas se ve favorecida si los analitos se encuentran presentes en una matriz de baja fuerza iónica. Para la elución de los analitos, lo más adecuado es utilizar un disolvente de elevada fuerza iónica. Adsorbentes típicos para interacciones iónicas son las resinas Chelex–100 y Metpac CC-1.

2.2 - Extracción en fase sólida con resinas quelatantes y sólidos adsorbentes

Los procedimientos de extracción en fase sólida, tanto en modo "off – line", como en modo "on – line" son los métodos más ampliamente utilizados para la preconcentración de metales traza en el agua de mar. Los sólidos adsorbentes empleados en estos procedimientos pueden ser polares o apolares. Resulta frecuente llamar adsorbentes de "fase reversa" a los materiales que son más hidrofóbicos que la muestra, utilizados frecuentemente para preconcentrar muestras acuosas. Por otra parte, los adsorbentes de "fase normal" son aquellos materiales más polares que la muestra, y se utilizan cuando la muestra es un disolvente orgánico que contiene los analitos de interés. Cuando se utilizan soportes hidrofóbicos, la retención de las especies iónicas metálicas requiere la formación de complejos hidrofóbicos. Esto puede lograrse mediante la adición de un reactivo quelatante a la muestra, o mediante la inmovilización del reactivo en el sólido adsorbente. Los agentes quelatantes más empleados para este fin son la 8–hidroxiquinolina (8–HQ) y el dietilditiocarbamato (DDTC).

La 8–hidroxiquinolina es un producto que reacciona con más de 60 iones metálicos en disoluciones homogéneas, formando complejos con diferentes constantes de estabilidad [115]. La afinidad de la 8–hidroxiquinolina por los metales de transición, al tiempo que rechaza los metales alcalinos y alcalinotérreos, ha hecho de este agente quelatante una elección muy popular para la preconcentración de metales traza en el agua de mar. Como resultado, este producto y sus derivados han sido inmovilizados con éxito en diversos soportes poliméricos e inorgánicos [116-123].

La inmovilización de compuestos orgánicos sobre soportes sólidos está, por lo general, destinada a modificar su superficie con determinados grupos funcionales que permitan una mayor selectividad en la extracción. La selectividad de las fases sólidas modificadas hacia ciertos iones metálicos depende de varios factores, como la actividad de los grupos cargados en la superficie, el tipo de interacción de los analitos con los grupos funcionales, y el tamaño de los compuestos orgánicos utilizados para modificar el sólido adsorbente. La extracción selectiva de un solo elemento frente a otros iones interferentes requiere emplear una fase sólida adecuada, capaz de exhibir una afinidad suficiente para enlazarse selectivamente a un determinado ion metálico.

La naturaleza y las propiedades del sólido adsorbente son muy importantes para la retención efectiva de las especies metálicas. La elección cuidadosa del adsorbente es, por lo tanto, crucial en el desarrollo de la metodología de SPE. En la práctica, los principales requisitos de un sólido adsorbente son: (1) la posibilidad de extraer un gran número de elementos traza en un amplio rango de pH (junto con la selectividad frente a los iones mayoritarios); (2) la adsorción y elución rápida y cuantitativa de los analitos, (3) una alta capacidad; (4) regenerabilidad, y (5) fácil accesibilidad de los analitos a los sitios de unión. Los adsorbentes que permiten velocidades de reacción rápidas, así como aquellos que poseen una capacidad de carga elevada, suelen ser los más empleados.

Existen diversas revisiones [124-126] en las que se describe, de un modo general, la técnica de extracción en fase sólida y los distintos tipos de sólidos adsorbentes que pueden emplearse para la preconcentración de metales traza en varios tipos de muestras (aguas naturales, muestras geológicas, muestras biológicas, etc…). Según Camel [124], los sólidos adsorbentes pueden clasificarse en sólidos orgánicos e inorgánicos. El adsorbente inorgánico más ampliamente utilizado es la sílica gel (SiO_2), aunque también pueden utilizarse otros óxidos inorgánicos, como la alúmina ($A1_2O_3$), el óxido de magnesio (MgO) y los óxidos de otras especies. Los adsorbentes orgánicos pueden dividirse, a su vez, en poliméricos y no poliméricos. A continuación se describe brevemente cada tipo de sólido adsorbente.

2.2.1 - Adsorbentes inorgánicos

- Sílica gel

El gel de sílice, también llamado "sílica gel", es una sustancia química de aspecto cristalino, porosa, inerte, insoluble en agua, no tóxica e inodora, de fórmula química SiO_2. La sílica gel es el sólido adsorbente más ampliamente utilizado, ya que no se hincha ni se deforma, al tiempo que posee una buena estabilidad mecánica y térmica. La superficie de la sílica gel se caracteriza por la presencia de grupos silanol, que son intercambiadores iónicos débiles y permiten la extracción de especies iónicas. La retención cuantitativa de los iones depende en gran medida del pH de la muestra, ya que en condiciones ácidas los grupos silanol se encuentran protonados y su capacidad intercambiadora de iones se ve muy reducida o, incluso, completamente impedida.

Este adsorbente posee una baja selectividad, y es propenso a la hidrólisis a pHs básicos. Por lo tanto, su superficie ha sido modificada en numerosas ocasiones para obtener sólidos adsorbentes con una mayor selectividad. Dos métodos que se emplean habitualmente para modificar la superficie de la sílica son la inmovilización química y

la adsorción física de determinados compuestos orgánicos. En el primer método se forma un enlace químico entre los grupos funcionales de la sílica y los compuestos orgánicos (adsorbente modificado), mientras que en el segundo, el compuesto orgánico es directamente adsorbido sobre la superficie de la sílica (adsorbente impregnado o cargado), ya sea pasando una disolución del reactivo a través de una columna rellena con el adsorbente, o mediante la inmersión del adsorbente en una disolución del reactivo.

Diversos reactivos han sido investigados para impregnar la sílica gel como medio para aumentar su capacidad de retención y su selectividad hacia determinados elementos traza. Los reactivos que se emplean habitualmente son intercambiadores iónicos o compuestos quelatantes [127-132]. Sin embargo, el soporte presenta una mayor estabilidad cuando el reactivo se enlaza químicamente a la sílica. Numerosos reactivos han sido inmovilizados con éxito sobre la sílica gel. Entre ellos se encuentran aminas [133], ditioacetales [134], ditiocarbamatos [135,136], y la 8-hidroxiquinolina (8-HQ) [116-119]. Su elección cuidadosa ha permitido realizar algunos estudios de especiación [137,138]. Sin embargo, es necesario tener en cuenta que, a pesar del enlace químico de distintos grupos funcionales sobre la superficie de la sílica, seguirán existiendo grupos silanol libres que podrían participar en la retención no selectiva de diversos elementos traza, sobre todo a pHs por encima de su pKa (forma ionizada), aunque su número puede reducirse mediante el recubrimiento del adsorbente (end-capping).

- Sílica C18

A pesar de la gran variedad de fases enlazadas disponibles, la C18 es, en la actualidad, la fase más ampliamente utilizada. Sin embargo, debido a su carácter hidrofóbico no resulta adecuada para la retención directa de elementos traza, siendo necesaria la adición de un ligando a la muestra antes de su paso a través del adsorbente. Entre los

reactivos empleados se pueden citar la 8-HQ [139-142], el pirrolidín ditiocarbamato amónico (APDC) [143], la 1,10-fenantrolina [144], o la batocuproina [145]. Otra posibilidad consiste en inmovilizar el reactivo sobre la C18 y complejar los metales pasando la muestra a través de esta fase. La C18 modificada con los ligandos apropiados ha sido utilizada con éxito para la separación y determinación de distintos iones metálicos en diferentes muestras de aguas [146,147]. Spivakov *et al.* [148] han publicado recientemente una revisión sobre la extracción en fase solida de elementos traza mediante el empleo de distintas fases enlazadas de sílica gel. Sin embargo, a pesar de su amplia aplicación a la preconcentración de elementos traza, las fases de sílica enlazadas presentan la desventaja de que solo pueden ser empleadas en un rango limitado de pH, pues a pHs ácidos (por debajo de 4) y a pHs básicos (por encima de 8) puede ocurrir la hidrólisis de los compuestos. En consecuencia, los adsorbentes poliméricos suelen ser más apropiados, ya que se pueden emplear en todo el rango de pH.

- Otros óxidos inorgánicos.

A parte de sílica gel, otros óxidos inorgánicos han sido empleados para la adsorción de elementos traza, como el dióxido de titanio (TiO_2), la alúmina (Al_2O_3), o el dióxido de circonio (ZrO_2). Mientras que el SiO_2 se emplea para adsorber cationes, debido a sus propiedades ácidas, los óxidos básicos, como el óxido de magnesio (MgO), se emplean para adsorber aniones. La adsorción de los iones sobre la superficie de los óxidos se atribuye a la participación de los grupos hidroxilo. Estos grupos se encuentran cargados negativamente en condiciones básicas (desprotonados), reteniendo así los cationes, y positivamente en condiciones ácidas (protonados), reteniendo así los aniones. Consecuentemente, en los óxidos anfotéricos (TiO_2, Al_2O_3, ZrO_2) los cationes son adsorbidos en condiciones básicas (pH por encima del punto isoeléctrico del óxido),

mientras que los aniones son adsorbidos en condiciones ácidas (pH por debajo del punto isoeléctrico del óxido). Por ejemplo, la especiación de cromo puede lograrse empleando alúmina ácida, mediante el ajuste cuidadoso del pH de la muestra: pH 2 para la retención de Cr (VI) y pH 7 para la retención del Cr (III) [149,150].

La adsorción en los óxidos inorgánicos también se puede ver influenciada por la presencia de sales en la matriz. En particular, las altas concentraciones de fosfatos y sulfatos pueden disminuir la retención de elementos traza sobre el óxido de titanio [151], mientras que los cationes mayoritarios (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}), son débilmente adsorbidos en este compuesto [151,152].

2.2.2 - Adsorbentes orgánicos

Los adsorbentes orgánicos pueden dividirse en poliméricos (polímeros naturales y sintéticos) y no poliméricos (carbón, naftaleno…). Los adsorbentes poliméricos han sido, con diferencia, los más utilizados para la preconcentración de elementos traza porque poseen la ventaja sobre la sílica gel modificada de que pueden ser empleados en todo el rango de pH.

La eficacia de los adsorbentes poliméricos depende de varios factores, como el tamaño de partícula, el área superficial, el diámetro de poro y el grado de entrecruzamiento. En la mayor parte de las aplicaciones, diversos grupos quelatantes han sido enlazados químicamente a las cadenas poliméricas, dando lugar a nuevos sólidos adsorbentes. La mayoría de los grupos quelatantes enlazados poseen una baja solubilidad en el agua para evitar que se desprendan del soporte, sin embargo, la adición de grupos demasiado hidrofóbicos no resulta adecuada, pues dificultan la humectación del adsorbente por la muestra acuosa, originando así una pobre eficacia de retención. Kantipuly *et al.* [153] han publicado en 1990 una revisión de los distintos soportes poliméricos existentes para la preconcentración de metales traza. A continuación se describe cada uno de ellos.

- Adsorbentes de poliestireno-divinilbenceno

Las Amberlitas XAD-1, XAD-2, XAD-4 y XAD-16 son resinas macroporosas de poliestireno-divinilbenceno (PS-DVB). Debido a su carácter hidrofóbico, la retención de elementos traza en estos adsorbentes requiere la adición de un ligando a la muestra, como el APDC [154], la 8-HQ [155,156], o la difenilcarbazida (DPC) [157]. Alternativamente, también se pueden asociar distintos ligandos al PS-DVB por adsorción física como la ditizona [158], el 3-(2-piridil)-5,6-difenil-1,2,4-triacina (PDT) [159], la tropolona [160,161], el 1-(2-piridilazo)-2-naftol (PAN) [162,163], el 7-dodecenil-8-quinolinol (DDQ) [164], el APDC [165] o el 2-(5-bromo-2-piridilazo)-5(dietilamino)fenol (5-Br-PADAP) [166]. Sin embargo, en la práctica, las resinas preparadas por impregnación del ligando son difíciles de reutilizar, debido a la lixiviación parcial del ligando tras la utilización del polímero, lo que origina una baja reproducibilidad de los análisis. Para solventar este problema, se han realizado diversas modificaciones químicas del PS-DVB [167]; sin embargo, sólo unas pocas se encuentran disponibles comercialmente.

- Polímeros de poliacrilato

La Amberlita XAD-7 y la Amberlita XAD-8 son resinas de etilen glicol-dimetacrilato (EGDMA). Poseen un carácter no-aromático y una capacidad de intercambio iónico muy baja. Estas resinas permiten la recuperación de compuestos polares, sin embargo, la mayoría de las veces se agregan reactivos quelatantes a la muestra, para aumentar la retención, debido a la moderada polaridad de los acrilatos [168]. Los reactivos quelatantes también han sido cargados en estas resinas, para aumentar su selectividad y su capacidad de retención de elementos traza. Los adsorbentes cargados son estables durante varios meses y pueden ser reutilizados [166,169-171], aunque los grupos quelatantes también pueden ser enlazados químicamente para lograr una mayor estabilidad de los compuestos [172,173]. Soylak *et al.* [174] han publicado en 2001 una revisión sobre las resinas de Amberlita XAD y su aplicación en los procesos de SPE.

- Polímeros de divinilbenceno-vinilpirrolidona

Los adsorbentes hechos de divinilbenceno-vinilpirrolidona (DVB-VPD) han sido desarrollados para su aplicación en la determinación de compuestos orgánicos en muestras de agua [175]. Sin embargo, por el momento, no han sido empleados para la preconcentración de elementos traza.

Su composición consiste en una proporción equilibrada de N-vinilpirrolidona (hidrofílica) y divinilbenceno (hidrofóbico). La N- vinilpirrolidona permite la apropiada humectación de la resina, mientras que el divinilbenceno permite la retención en "fase reversa" de los analitos. Estos polímeros presentan la ventaja, en comparación con los adsorbentes clásicos, de que pueden secarse durante el procedimiento de extracción, sin reducir su capacidad para retener los analitos. Además, son estables a cualquier pH.

Dos polímeros de divinilbenceno-vinilpirrolidona comerciales son los cartuchos Oasis HBL y MCX (Waters, Milford, MA, USA).

- Polímeros de poliuretano.

La espuma de poliuretano (Polyurethane Foam, PUF) ha sido empleada en diversos procesos de SPE debido a su capacidad de adsorción de varios elementos traza. Sin embargo, la mayor parte de las veces se recubre con un reactivo complejante como la dimetilglioxima (DMG) [176], el 1-nitroso-2-naftol (NN) [177], el dietil-ditiocarbamato DDTC [166] o el hexametilen-ditiocarbamato (HMDC) [178] para mejorar su capacidad de adsorción.

El reactivo quelatante también puede añadirse directamente a la muestra, y los quelatos metálicos formados se retienen posteriormente en la espuma de poliuretano, como se ha llevado a cabo con los complejos del tiocianato [179-181] y del O,O dietil-ditiofosfato (DDTP) [182].

- Polímeros de polietileno.

El polietileno también ha sido empleado para la preconcentración de elementos traza porque es un soporte que adsorbe varios complejos metálicos hidrofóbicos. Además, los complejos adsorbidos pueden ser eluidos con un pequeño volumen de disolvente orgánico lo que permite elevados factores de preconcentración.

El polietileno puede ser empleado en medios fuertemente ácidos y básicos, por esta razón, se ha utilizado como adsorbente para la retención de cromo en un medio ácido después de la adición de DPC [183].

- Polímeros de politetrafluoroetileno

Los polímeros de politetrafluoroetileno (PTFE) pueden retener los elementos traza después de la adición de un reactivo quelatante a la muestra como el APDC [184-187] o la ditizona (DZ) [188]. También pueden ser recubiertos con un ligando adecuado, como la 2-metil-8-hidroxiquinolina [189].

- Polímeros de poliestireno

Los polímeros de poliestireno pueden ser una interesante alternativa a los adsorbentes convencionales cuando tienen una estructura muy entrecruzada. Se trata de absorbentes hidrofóbicos, por lo que se requiere la adición de un reactivo a la muestra para formar complejos que son posteriormente retenidos en los polímeros [190].

- Polímeros de poliamida.

Los polímeros de poliamida se han utilizado para la retención de elementos de las tierras raras, mediante la adición de un reactivo quelatante a la muestra para complejar los elementos traza en disolución [191].

- Resinas quelatantes iminodiaceticas

Entre los distintos soportes existentes, las resinas con grupos funcionales ácido iminodiacetico [$-CH_2-N(CH_2COO^-)_2$] han sido las más empleadas para la determinación de metales traza en el agua de mar.

La resina más ampliamente utilizada desde que su uso fue introducido por Riley y Taylor [192] en 1968, es la Chelex–100 [193-201]. Esta resina, que se obtiene enlazando los grupos funcionales iminodiaceticos a un polímero de poliestireno, muestra una fuerte preferencia por los metales de transición sobre los metales alcalinos y alcalinotérreos, y se puede adquirir comercialmente a un precio asequible. Sin embargo, la Chelex–100 presenta grandes variaciones de volumen, debido a los cambios que se producen en su forma iónica, a medida que varía el pH de las disoluciones que se introducen en la columna, lo que puede causar dificultades para mantener una velocidad de flujo razonable a través de la columna. Otras resinas con el mismo grupo funcional que la Chelex–100, como la Metpac-CC1 (Dionex) y la Muromac-A1 (Muromachi Chemicals, Tokyo) no sufren variaciones de volumen debido al substrato macroporoso de vinilbencil–divinilbenceno en el que el grupo funcional ha sido inmovilizado, que es menos susceptible que el poliestireno a estos cambios de volumen [202]. Ambas columnas se encuentran disponibles comercialmente y han sido utilizadas con éxito en diversas ocasiones para la determinación de metales traza en agua de mar [203-210].

El mayor problema que presentan las resinas iminodiaceticas es que el grado de protonación puede afectar de forma crítica a su capacidad de retener los cationes metálicos debido al débil carácter ácido del grupo funcional. Para la Chelex-100, por ejemplo, la protonación de los grupos carboxilato y del átomo de nitrógeno es completa a un pH de 2,21, mientras que la forma completamente desprotonada se alcanza a un pH de 12,30.

- Resinas quelatantes de propilendiaminotetraacetato

Las resinas quelatantes de propilendiaminotetraacetato son polímeros macroporosos basados en el ácido propilendiaminotetraacético (PDATA) que poseen una estructura muy similar a la del ácido etilendiaminotetraacético (EDTA), permitiendo la retención de varios elementos traza, mediante quelación [211].

- Resinas basadas en el poliacrilonitrilo

Las fibras de poliacrilonitrilo han sido modificadas para obtener adsorbentes quelatantes con grupos aminofosfónicos [212], ditiocarbamatos [212] o aminotioureas [213]. Sin embargo, como las síntesis son laboriosas, una alternativa es cubrir las fibras de poliacrilonitrilo con un reactivo apropiado, como la 8-HQ [214,215], para la posterior retención de los elementos traza.

- Adsorbentes de carbono.

El carbón activado es un derivado del carbón extremadamente poroso. Se prepara mediante la oxidación del carbón vegetal a baja temperatura. Debido a su gran área superficial (300-1000 m^2/g), se emplea para la adsorción de compuestos orgánicos y elementos traza, mediante de fuerzas de Van der Waals y puentes de hidrógeno a través de los grupos polares del compuesto (carbonilos, hidroxilos y carboxilos) [216]. Por lo tanto, los elementos traza pueden ser directamente adsorbido en carbón activado [217], o pueden ser retenidos después de la adición de un agente quelatante a la muestra, como los aminoácidos [218], la ditizona [219], el APDC [220,221], el PAN [222], la 8-HQ [223], el cupferrón [223], el Bismutiol II [224] o el DDTP [182]. El ligando elegido no debe interaccionar fuertemente con el carbón activado, de otro modo podría observarse la completa disociación del quelato metálico [216].

El principal inconveniente al utilizar carbones activados es su superficie heterogénea, con diversos grupos funcionales activos, lo que a menudo produce bajas reproducibilidades. Además, estos adsorbentes son muy reactivos y pueden actuar como catalizadores en procesos de oxidación y otras reacciones químicas. Afortunadamente, junto con el desarrollo de materiales poliméricos y fases enlazadas, se ha desarrollado una nueva generación de absorbentes de carbono con una estructura más homogénea y propiedades más reproducibles: los negros de carbón grafitizados.

Los negros de carbón grafitizados (Graphitized Carbón Blacks, GCB) se obtienen calentando negros de carbón a 2700-3000 ºC en una atmósfera inerte [225]. Son adsorbentes no específicos, poco porosos (área superficial aprox. 100 m^2/g), que se consideran intercambiadores aniónicos y adsorbentes de fase reversa debido a la presencia de heterogeneidades químicas con carga positiva en su superficie. Estos adsorbentes han sido ampliamente utilizados para la SPE de contaminantes orgánicos polares en muestras de agua [225], pero su uso para la preconcentración de elementos traza es todavía escaso [226]. Sus principales inconvenientes son la posible retención irreversible de los analitos, y una pobre estabilidad mecánica.

- Celulosa

La celulosa ha sido eficazmente empleada para la retención de elementos traza en muestras de agua, ya sea directamente o tras la adición de un agente quelatante a la muestra [227,228]. En particular, ha permitido realizar la especiación de cromo mediante la retención selectiva de Cr (III) [227]. Este adsorbente también puede ser modificado, para aumentar la selectividad de la SPE. Así, ha sido posible la especiación del selenio sobre la celulosa modificada con una amina cuaternaria, debido a la elución selectiva del Se (IV) y el Se (VI), empleando acido nítrico en dos concentraciones diferentes [229].

- Adsorbentes basados en el naftaleno

La retención de elementos traza en naftaleno microcristalino también es factible, ya sea después de la adición de un ligando a la muestra [230], o después de realizar la modificación del sólido, para garantizar mejores características de adsorción [231,232]. Sin embargo, el uso de este soporte es bastante poco común. Además, por el momento, únicamente ha sido empleado en experimentos “en baño”.

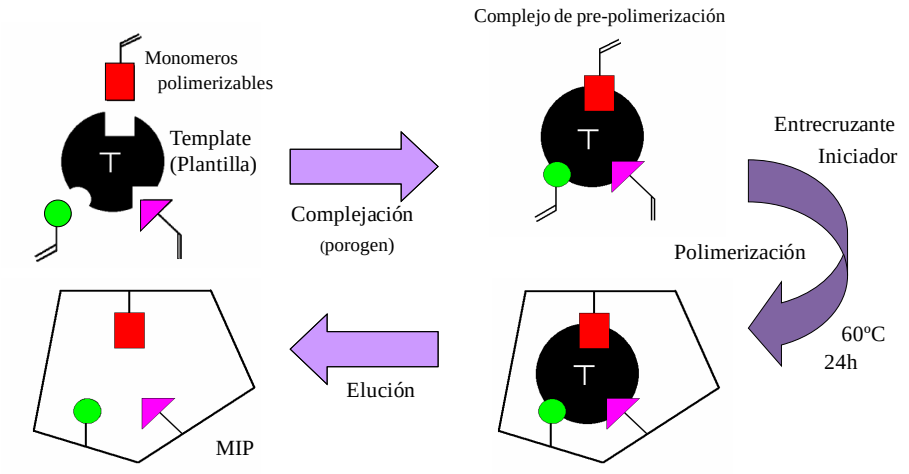
2.3 – Extracción en fase solida mediante polímeros de impronta molecular

La técnica de la impronta molecular se basa en la preparación de un polímero altamente entrecruzado alrededor de un analito "plantilla" (template), en presencia de un monómero adecuado [233]. En primer lugar es necesario poner el analito en contacto con el monómero en un disolvente adecuado con el objetivo de que se forme un complejo de pre-polimerización estable. A continuación, se realiza la polimerización en presencia del entrecruzante. Por último, el polímero debe ser sometido a un proceso de limpieza para eliminar al analito de las cavidades formadas, que serán capaces, posteriormente, de retener selectivamente el analito en una muestra compleja.

Mediante la tecnología de impronta molecular es posible sintetizar polímeros muy estables que presentan sitios de reconocimiento en la matriz polimérica complementarios en tamaño y forma con el analito para el que fueron generados, presentando constantes de afinidad comparables a las del reconocimiento antigénico [234]. Una de las mayores ventajas que presentan estos polímeros, en comparación con los anticuerpos, es la sencillez y bajo coste de su síntesis. Debido a estas ventajas, el interés por los polímeros de impronta molecular (Molecularly Imprinted Polymers, MIPs) ha ido en aumento en los últimos años. Por el momento, los MIPs han sido empleados fundamentalmente como materiales selectivos para cromatografía y procesos de extracción en fase sólida, en los cuales, sus características en términos de afinidad y selectividad son suficientes para sustituir a los anticuerpos [235].

En la Figura 1 está representada de forma esquemática la técnica de la impronta molecular.

Figura 1. Esquema de la preparación de un polímero de impronta molecular.



2.3.1 - Síntesis de polímeros de impronta molecular

Los factores que influyen durante el proceso de síntesis de los polímeros de impronta molecular, así como los métodos de polimerización y su aplicación como fases estacionarias para procedimientos de extracción en fase sólida se encuentran excelentemente resumidos en las revisiones que Antonio Martin-Esteban ha publicado durante los últimos años [233,235,236]. Según Martin-Esteban [233], las características (selectividad, afinidad, capacidad…) y propiedades físicas del polímero obtenido (porosidad, rigidez, área superficial…) van a estar directamente relacionadas con la estructura y los grupos funcionales que presente el analito plantilla, así como con el monómero, el entrecruzante y el disolvente en el que se lleve a cabo la polimerización (porogen). A continuación se describe brevemente cada uno de ellos.

- Analito y monómeros

Por lo general, los MIPs son capaces de distinguir entre la molécula para la que fueron generados y moléculas muy parecidas, si estas presentan alguna pequeña diferencia

estructural o conformacional (isómeros). Por este motivo, la forma y el tamaño del analito plantilla van a tener una gran influencia en la selectividad que presente el polímero posteriormente.

El monómero ha de contener en su estructura grupos funcionales capaces de interaccionar con el analito con energía suficiente para generar un complejo que se mantenga estable durante la polimerización. La formación de este complejo puede realizarse mediante una unión covalente o mediante uniones no covalentes, que impliquen interacciones electrostáticas, puentes de hidrogeno o enlaces de coordinación con iones metálicos.

El primer trabajo sobre impronta molecular mediante enlace covalente, fue publicado por Wulff y Sarhan en 1972 [237]. En él se describe la preparación de un polímero cuyas cavidades presentan propiedades quirales que permiten la separación de especies en una mezcla racémica. Por lo general, la técnica de impronta molecular por formación de enlaces covalentes permite una gran definición estructural en la forma y tamaño de la cavidad generada por el analito. Sin embargo, resulta poco versátil, ya que existen pocos monómeros y analitos que presenten grupos funcionales apropiados para formar dichos enlaces.

Mosbach et al. [238] fueron los primeros en emplear interacciones no covalentes como base para la impronta molecular en 1981. En este caso, existe un número mucho mayor de monómeros capaces de interaccionar con los analitos, lo que ha permitido aplicar la impronta molecular a diferentes tipos de compuestos. Por este motivo, el interés por la técnica ha ido en aumento en los últimos años, y muchos han sido los trabajos publicados que versan sobre diferentes aspectos a cerca de la tecnología de impronta molecular [239,240]. En cualquier caso, independientemente del monómero elegido, resulta recomendable emplear una gran cantidad de monómero con el fin de desplazar el

equilibrio hacia la formación del complejo de prepolimerización. Por lo general, una relación molar 1:4 (plantilla:monómero) es suficiente para lograr nuestro objetivo. Sin embargo, el exceso de monómero libre tras la polimerización puede provocar la presencia de sitios de unión no específicos en el polímero, lo que hace necesario optimizar las disoluciones de carga, lavado y elución del analito [233].

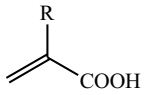
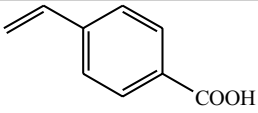
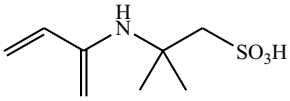
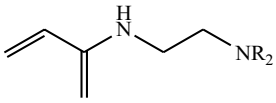
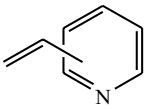
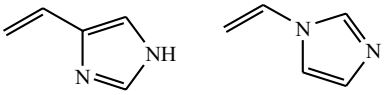
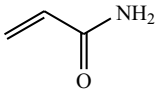
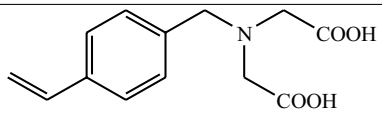
El ácido metacrílico (MAA) ha sido el monómero más empleado hasta la fecha, resultando muy apropiado para aquellos analitos que presentan grupos funcionales básicos en su estructura. Del mismo modo, la vinilpiridina (VP) resulta muy adecuada para analitos que contengan grupos ácidos en su estructura. Otros monómeros también empleados, aunque en menor extensión, se muestran en la Tabla 7 [234].

- Entrecruzante y porogen

La morfología del polímero va a venir determinada tanto por las propiedades del disolvente (porogen) como del entrecruzante (cross-linker). Ambos tienen una influencia conjunta en las propiedades físicas y químicas del polímero obtenido, particularmente en su porosidad, la cual ha de ser suficiente para permitir el acceso del analito a los sitios de unión generados, aunque en algunos casos, la porosidad del polímero puede variar en los tratamientos posteriores a la polimerización.

Las interacciones que se producen entre el monómero y la plantilla van a estar muy influenciadas por el disolvente empleado durante la polimerización. Por lo general, la estabilización de los enlaces por puentes de hidrógeno, o por interacciones electrostáticas entre los monómeros y la plantilla, se va a ver muy favorecida mediante el empleo de disolventes con una constante dieléctrica baja, como el tolueno o el cloroformo. Los polímeros obtenidos al utilizar otros disolventes, con mayores constantes dieléctricas (por ejemplo, acetonitrilo) presentan, generalmente, una menor afinidad en el reconocimiento posterior del analito.

Tabla 7. Monómeros y tipos de interacciones empleados para la impronta molecular de polímeros por enlaces no covalentes.

Monómero	**Estructura**	**Tipo de interacción**
Ácidos acrílicos		- Enlaces de hidrógeno - Iónicas
Ácidos vinilbenzoicos		- Enlaces de hidrógeno - Iónicas
Ácidos acrilamido sulfónicos		- Iónicas
Aminometil-acrilamidas		- Iónicas
Vinilpiridinas		- Iónicas - Enlaces de hidrógeno - Transferencia de carga
Vinilimidazoles		- Iónicas - Enlaces de hidrógeno - Coordinación
Acrilamidas		- Enlaces de hidrógeno
Ácidos vinil-iminodiaceticos		- Coordinación

El uso de disolventes más polares, como el agua o el metanol, no es recomendable puesto que van a competir con el analito por la formación de puentes de hidrógeno con el monómero, interfiriendo significativamente en la formación de los enlaces con la plantilla.

Una vez formado el complejo, es necesario preservar su integridad estructural durante el proceso de polimerización con el fin de generar cavidades complementarias en tamaño y forma al analito plantilla. Para ello resulta necesario conferir al polímero un alto grado de entrecruzamiento, de modo que la fijación espacial de los grupos funcionales en la matriz polimérica se lleve a cabo de una forma estable [233].

El etilen glicol-dimetacrilato (EGDMA) es el entrecruzante más comúnmente empleado en combinación con el MAA, resultando muy adecuado ya que preserva los sitios de unión durante la polimerización, al mismo tiempo que confiere al polímero una gran estabilidad mecánica y térmica, buena mojabilidad y transferencia de masa. Únicamente el trimetil propano trimetacrilato (TRIM) posee características similares, y en algún caso mejores, que el EGDMA.

- Polimerización

Como se indica anteriormente, la primera etapa en el proceso de síntesis consiste en la formación del complejo plantilla:monómero en un pequeño volumen del porogen apropiado, al que posteriormente se le añade el entrecruzante. En los sistemas basados en el MAA como monómero, y el EGDMA como entrecruzante, la relación molar más adecuada es 1:4:20 (plantilla:monómero:entrecruzante) [233].

La polimerización se inicia añadiendo un iniciador de tipo azo, normalmente 2,2´- azo-bis-isobutironitrilo (AIBN), y sometiendo la mezcla a una temperatura igual o superior a 60ºC o a una radiación ultravioleta (a 15ºC). Por lo general, la polimerización se lleva a cabo durante 24 horas.

El proceso de polimerización más ampliamente utilizado es la polimerización en bloque (bulk polymerization). Mediante este proceso se obtienen polímeros de forma idéntica al recipiente empleado para realizar la polimerización (generalmente un tubo de ensayo), el cual ha de romperse para recuperar el polímero. Posteriormente es necesario triturarlo y tamizarlo hasta el tamaño de partícula deseado (25-38 μm para cromatografía, o 50-200 μm para extracción en fase solida). Este tipo de polimerización es el método más sencillo y barato. Sin embargo, presenta varios inconvenientes; por un lado, las partículas obtenidas tras la trituración y tamizado no son homogéneas en cuanto a tamaño y forma; por otro lado, tras el proceso completo la cantidad de polímero que puede ser usado con fines analíticos se reduce a menos del 50% del inicial. Además, una parte de los sitios específicos generados se destruyen en el proceso de trituración. Todo ello conlleva una pérdida importante de la capacidad de carga del polímero. Aunque las partículas obtenidas mediante el proceso de polimerización en bloque resultan apropiadas para SPE, son necesarios varios tratamientos antes de poder empaquetarlas en una columna cromatográfica.

Otro tipo de polimerizaciones permiten obtener el polímero en forma de partículas más o menos esféricas, como es el caso de la polimerización por dispersión [241] o por precipitación [242,243]. En ambos métodos se emplea un gran volumen de disolvente, diferenciándose en la manera de estabilizar las partículas formadas. En los métodos de polimerización por dispersión se utilizan estabilizantes o surfactantes que encapsulen las partículas formadas para su posterior separación, mientras que en la polimerización por precipitación la rigidez y entrecruzamiento de las partículas formadas es suficiente para permitir su separación. Las partículas obtenidas mediante estas técnicas son muy homogéneas en tamaño y forma, sin embargo, resulta difícil establecer unas reglas generales que permitan la obtención de partículas con un tamaño concreto.

Otra opción consiste en la polimerización sobre la superficie de partículas esféricas de sílica, o de politrimetil propano trimetacrilato [244]. En este caso, el polímero se obtiene en forma de partículas mixtas, cuyo interior es el material empleado como soporte, por lo que solo la superficie de la partícula obtenida tiene las propiedades de reconocimiento adecuadas. También es posible realizar la polimerización en los poros de estos materiales, de modo que el tamaño y la forma de las partículas obtenidas se corresponderán con el de los poros del material empleado [245,246].

En la Tabla 8 se recogen otros métodos existentes en la bibliografía, para la obtención de polímeros en diferentes formatos. Un buen resumen de los diferentes métodos de polimerización existentes, se encuentra en las revisiones realizadas por Kaavi y Pichon [247] y Arshady [248].

- Limpieza

Una vez obtenido el polímero con el tamaño de partícula deseado es necesario someterlo a un proceso exhaustivo de limpieza, con el objetivo de extraer el analito plantilla de la matriz polimérica. Generalmente estos procesos se llevan a cabo mediante lavados del polímero con disolventes capaces de destruir las interacciones plantilla:monómero, o mediante procesos de extracción Soxhlet, aunque en la mayoría de los casos resulta prácticamente imposible la eliminación total del analito plantilla de la matriz polimérica, lo que puede provocar la aparición de residuos del mismo durante las etapas de elución en procesos de extracción que se lleven a cabo con el polímero.

Tabla 8. Otros métodos de polimerización presentes en la bibliografía.

Método de polimerización	**Formato**	**Ref.**
Suspensión en agua	Partículas esféricas polidispersas	[249-251]
Suspensión en perfluorocarbono	Partículas esféricas polidispersas	[252]
Polimerización en dos etapas	Partículas esféricas monodispersas	[253-256]

2.3.2 - Extracción en fase sólida sobre MIPs (MIP-SPE)

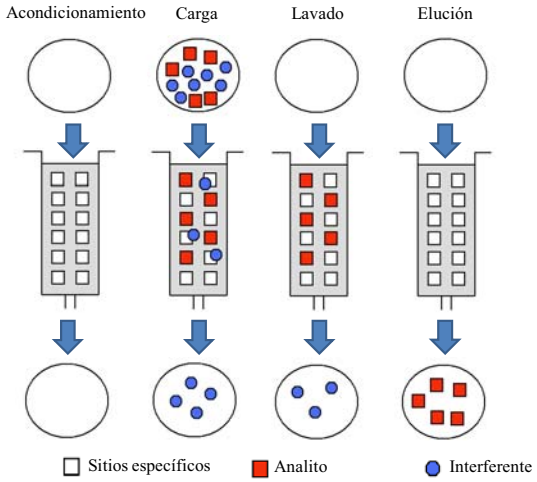
El primer estudio de extracción en fase sólida sobre un polímero de impronta molecular fue realizado por Sellergreen en el año 1994 [257]. Los procesos de extracción en fase sólida sobre MIPs pueden llevarse a cabo en modo "off-line" y en modo "on-line", sin embargo, la mayor parte de los trabajos publicados hasta el momento han sido desarrollados en modo "off-line", debido a que resulta más sencillo y económico.

El modo "on-line" presenta la ventaja de que no es necesario manipular la muestra entre la etapa de preconcentración y la etapa de análisis, de modo que se reduce la posibilidad de que se pierda una parte del analito, al tiempo que se reduce el riesgo de contaminación de la muestra. Otras ventajas que presenta esta modalidad es que el volumen de muestra necesario es más pequeño, el consumo de disolventes menor y resulta más fácil automatizar el procedimiento. Los límites de detección y la reproducibilidad también mejoran al emplear este modo.

El proceso de extracción en fase sólida sobre MIPs es idéntico a cualquier otro procedimiento de extracción en fase sólida. En primer lugar es necesario trasvasar una pequeña cantidad del polímero (generalmente 50-200 mg) a una columna (extracciones en continuo) o a un cartucho (extracciones en discontinuo) y, a continuación, es necesario acondicionar el polímero con el disolvente en el que, posteriormente, se realizará la carga de la muestra. Tras la fijación selectiva de los analitos se llevará a cabo un proceso de lavado, para eliminar aquellos compuestos interferentes que hayan podido quedar retenidos de forma inespecífica en la matriz polimérica y, por último, los analitos serán eluidos convenientemente.

El proceso de extracción sobre un polímero de impronta molecular se muestra en la Figura 2.

Figura 2. Proceso de extracción sobre un polímero de impronta molecular.



Una parte de los analitos de interés puede quedar retenida en la matriz polimérica mediante interacciones no-específicas que se producen habitualmente debido al exceso de monómero empleado durante el proceso polimerización. La manera más sencilla de evaluar estas interacciones consiste en realizar un estudio de recuperación sobre un polímero blanco (Non Imprinted Polymer, NIP), que se obtiene mediante un proceso de síntesis similar al del MIP, pero sin adicionar la plantilla a la mezcla inicial, de forma que las matrices de ambos polímeros sean similares. En algún caso, también se ha utilizado como blanco un polímero preparado para algún compuesto similar al analito de interés [258].

- Carga de la muestra

La mayoría de los polímeros empleados hoy en día se basan en interacciones del tipo "puente de hidrogeno". Por lo tanto, en este tipo de polímeros, el disolvente más adecuado para la carga de la muestra será aquel que favorezca la formación de este tipo de uniones, mientras que el disolvente de elución más adecuado será aquel que nos

permita destruir los enlaces formados. De un modo general, se puede decir que el disolvente más adecuado para realizar la carga es aquel en el que se llevó a cabo la polimerización (es decir, el porogen) pues en él se reproduce el entorno donde se sintetizó el polímero, favoreciendo la formación de uniones especificas entre los grupos funcionales del analito y del monómero.

- Lavado

Con el objetivo de conseguir una buena limpieza de la muestra resulta necesario realizar una etapa de lavado previa a la elución, empleando algún disolvente que permita destruir las interacciones no-específicas de los compuestos presentes en la matriz de la muestra, sin perder los analitos de interés.

- Elución

Una vez que los compuestos interferentes han sido eliminados de la matriz polimérica, podemos eluir los analitos retenidos mediante la adición de un disolvente adecuado (polar, prótico, un ácido o una base). De esta forma, se destruyen los enlaces formados entre los analitos y los monómeros presentes en las cavidades del polímero.

La etapa de elución es determinante en el tiempo de vida del polímero, pues los cambios en la polaridad de los disolventes empleados pueden provocar variaciones en su morfología (contracciones o hinchamientos de la matriz polimérica), que pueden afectar a la accesibilidad de los sitios de unión [259]. Después de numerosas extracciones, la contracción de los huecos puede provocar una falta total de reconocimiento del analito plantilla [258].

Otro problema asociado a la etapa de elución se debe al analito plantilla que no ha sido eliminado de la matriz polimérica tras el proceso de síntesis. La extracción de la plantilla tras la polimerización raramente es totalmente efectiva, lo que puede originar contaminaciones aleatorias durante las etapas de elución (leaking) y, por tanto, errores

considerables en las determinaciones a niveles traza. Para evitar estas contaminaciones se ha realizado una serie de pruebas, sometiendo el polímero a procesos de limpieza más agresivos o a elevadas temperaturas [260], que no han dado buenos resultados, debido a los problemas relacionados con los cambios en la morfología del polímero.

Un procedimiento empleado para corregir las contaminaciones aleatorias en los procesos de elución ha sido la extracción paralela de una disolución "blanco" sobre otro cartucho que contenga la misma cantidad de polímero [261]. La determinación final se realiza por diferencia de las concentraciones encontradas en la muestra y en el blanco, para lo cual resulta necesario asumir que las contaminaciones van a ser las mismas en ambos cartuchos. Otra opción empleada, ha sido la preparación de polímeros utilizando como plantilla un compuesto estructuralmente análogo al analito que se quiere determinar, de tal forma que la posible contaminación no afecte a la cuantificación del analito de interés [262,263]. Estos materiales, sin embargo, han resultado ser menos selectivos que los polímeros de impronta convencionales [258].

2.3.3 - Aplicación de los polímeros de impronta molecular

Durante los últimos años se ha sintetizado un gran número de polímeros de impronta molecular para la preconcentración de distintos analitos: herbicidas [264,265], fenoles [266-268], antibióticos [269,270], drogas [271,260], etc.... La variedad de muestras en las que los analitos han sido extraídos también es muy elevada, e incluye sangre [272,273], orina [274,275], alimentos [276-278], pelo [279,280], y diversos tipos de muestras medioambientales como agua de río [281,282], agua de mar [283], muestras de suelos [284,285] y vegetales [264]. Un excelente resumen de las aplicaciones de los polímeros de impronta molecular como soportes para extracción en fase sólida, tanto en modo "on-line", como en modo "off-line" se encuentra en las revisiones de Quiao *et al.* [286], Lasakova *et al.* [287] y Kloskowski *et al.* [288].

2.4 - Extracción en fase solida mediante polímeros de impronta iónica

Los Polímeros de Impronta Iónica (Ionic Imprinted Polymers, IIPs) son similares a los MIPs, pero reconocen iones metálicos después de la impronta. Durante los últimos años se han sintetizado un gran número de IIPs para el reconocimiento de lantánidos [289-293], actínidos [294-298], metales nobles [299,300] y elementos de transición como el cobre [301-303], el cadmio [304-306], el selenio [307], el níquel [308-310], el aluminio [311,312], el zinc [313], el plomo [314], el hierro [315,316], el cromo [317] y el mercurio [318].

Un excelente resumen sobre los polímeros de impronta iónica y su aplicación como fases estacionarias en procedimientos de extracción en fase sólida se encuentra en las revisiones que Prasada-Rao *et al.* [319,320] han publicado durante los últimos años. Según estos autores [320] se pueden establecer cuatro métodos para la síntesis de IIPs:

1) el entrecruzamiento de reactivos bifuncionales con polímeros de cadena lineal
2) la inmovilización química de ligandos vinilados en la matriz polimérica
3) la impronta en superficies mediante polimerización por emulsión
4) la captura de un agente quelatante no vinilado dentro de la matriz polimérica

Los tres primeros métodos emplean reactivos vinilados que forman complejos con la plantilla (el ion metálico) y pueden polimerizar a través de los grupos vinilo. En estos casos, el ligando complejante se encuentra químicamente inmovilizado en la matriz polimérica, mientras que en la técnica de captura, el ligando (un reactivo no vinilado) permanece atrapado dentro de la matriz del polímero pero no queda unido químicamente a las cadenas poliméricas. El método de captura ha sido aplicado con éxito para sintetizar una gran variedad de IIPs, empleando diferentes reactivos no vinilados como la dimetilglioxima [299], el diazoaminobenceno [318] y diversos derivados de la 8-HQ, como el 5,7-dicloro-8-HQ [290-293].

2.5 - Análisis "on-line"

Durante los últimos años, se han desarrollado varios métodos de extracción en fase sólida "on-line" para la preconcentración de elementos traza en el agua de mar con diferentes sólidos adsorbentes [321-324]**.** Estos métodos funcionan mediante el empleo de un colector distribuidor conectado en serie al instrumento de Espectroscopía Atómica con el que se realiza la determinación. Los métodos "on-line" presentan varias ventajas sobre los métodos tradicionales "off-line", así como ciertas desventajas. Las mejoras más importantes son, en general, la posibilidad de trabajar en un sistema cerrado, lo que conlleva una reducción significativa de la contaminación por partículas suspendidas en el aire, la reducción de los volúmenes de reactivos, la mejora de la reproducibilidad y la mayor facilidad para la automatización del sistema. Otra ventaja que presentan los métodos de preconcentración "on-line", todavía no desarrollada en toda su extensión, es la posibilidad de realizar la preconcentración de los analitos in-situ, bajo condiciones suaves (sin la necesidad de acidificar las muestras), minimizando las transformaciones químicas y biológicas que sufren los analitos, al tiempo que se reduce el contacto físico del personal investigador con las muestras, reduciendo así el riesgo de contaminación. A pesar de que las mejoras que representan estos métodos sobre los métodos "off-line" son evidentes, los métodos "on-line" inducen otros problemas, el principal de ellos surge cuando la determinación se realiza mediante ICP-OES o ICP-MS, puesto que la etapa de preconcentración puede llevar varios minutos en los que el plasma debe permanecer encendido, esperando la llegada de la muestra. Esto produce un encarecimiento del análisis debido al derroche del gas que genera el plasma (normalmente argon). A pesar de que, en general, los métodos de preconcentración "on-line" son más adecuados para trabajar con metales en agua de mar, los métodos "off-line" suelen ser más económicos y, actualmente, siguen siendo los más utilizados.

3 – Técnicas espectroscópicas empleadas para la determinación de metales traza en agua de mar

3.1 – Principios de la Espectroscopía Atómica

La Espectroscopía Atómica se basa en la medida de la radiación electromagnética que es absorbida o emitida por los átomos de una muestra. Mediante la espectroscopía atómica resulta posible la obtención de información cualitativa y cuantitativa de la muestra. En general, la información cuantitativa (concentración) se relaciona con la cantidad de radiación electromagnética que es emitida o absorbida, mientras que la información cualitativa (elementos presentes) se relaciona con las longitudes de onda a las que la radiación es emitida o absorbida.

En las técnicas de Espectrometría Atómica más comúnmente utilizadas en el análisis de elementos traza, la muestra es descompuesta por energía térmica, dando lugar a la formación de átomos en fase gas.

En la Espectrometría de Absorción Atómica (AAS), se hace incidir una radiación de longitud de onda característica a través del vapor atómico. Esta radiación es parcialmente absorbida por los átomos de ese elemento. La cantidad de luz que es absorbida por los átomos se mide, y permite cuantificar la concentración del elemento en la muestra.

En la Espectrometría de Emisión Atómica (AES), la muestra se somete a temperaturas lo suficientemente altas como para provocar no solo la atomización de la muestra, sino también la excitación de los átomos. Una vez que los átomos se encuentran en estados excitados, pueden devolver el exceso de energía mediante choques o emisión de radiación. En AES, se mide la intensidad de radiación emitida a longitudes de onda específicas y se relaciona con la concentración de los átomos del elemento.

En la Espectrometría de Fluorescencia Atómica (AFS), la fuente de radiación se emplea para excitar selectivamente los átomos del elemento de interés.

Cuando los átomos excitados devuelven el exceso de energía mediante emisión de radiación, se mide la energía emitida y se determina la concentración de igual modo que en AES. La excitación selectiva que se realiza en la técnica de AFS produce menos interferencias que en la técnica de AES. Sin embargo, resulta difícil detectar un gran número de elementos en una sola determinación, ya que el número de fuentes de excitación y detectores que se pueden disponer al mismo tiempo en el equipo es limitado.

3.1.1 - Atomización y fuentes de excitación

En general, existen tres tipos de fuentes térmicas usadas normalmente en espectrometría atómica analítica para atomizar la muestra: llamas, hornos y descargas eléctricas. Los dos primeros tipos de fuentes, las llamas y los hornos son lo suficientemente calientes para atomizar la muestra dando átomos libres. Las principales excepciones son los elementos que forman carburos u óxidos refractarios, que pueden existir como moléculas incluso a las temperaturas más elevadas de llamas y hornos (3000 – 4000 K). Debido a que la mayoría de los átomos libres en llamas y hornos se encuentran en su estado fundamental, la absorción atómica es el método más empleado para detectar la presencia del átomo de interés. Las excepciones, son aquellos elementos cuyo nivel excitado más próximo al fundamental es de energía lo suficientemente baja como para ser poblado fácilmente en una llama u horno. Como ejemplos, se pueden citar elementos tales como el litio, el sodio y el potasio. De hecho, la Espectrometría de Emisión en Llama es todavía la técnica más empleada para la detección de elementos alcalinos.

Las descargas eléctricas son el tercer tipo de fuentes de atomización usadas en Espectrometría Atómica analítica. Durante muchos años las únicas fuentes utilizadas en Espectrometría de Emisión fueron los arcos de corriente alterna y las chispas de corriente continua. Estas descargas eléctricas se crean por corrientes aplicadas o

potenciales alrededor de un electrodo en un gas inerte, y producen temperaturas de aproximadamente 7500 K. Actualmente, otros tipos de fuentes, como el plasma, se emplean como fuentes de atomización / excitación en AES.

3.1.2 - Comparación entre las diferentes técnicas espectroscópicas

La mayoría de los métodos empleados para la determinación de metales traza en el agua de mar utilizan la Espectroscopia Atómica como técnica analítica de detección. Las técnicas más ampliamente utilizadas son la Espectroscopía de Absorción Atómica por Llama (FAAS), la Espectroscopía de Absorción Atómica por Atomización Electrotérmica (ETAAS), la Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente (ICP-OES) y la Espectrometría de Masas por Plasma Acoplado Inductivamente (ICP-MS).

Las técnicas de llama y cámara de grafito presentan la ventaja inicial de ser sencillas de manejar y poseer un bajo coste instrumental. Una ventaja adicional del horno de grafito sobre la llama y el plasma es su alta sensibilidad, que permite obtener límites de detección significativamente más bajos para la mayor parte de los elementos. Otra ventaja del horno es la facilidad de análisis de pequeños volúmenes de muestra.

Las principales ventajas del ICP sobre las técnicas de absorción son: su capacidad para realizar análisis multielementales, su amplio rango dinámico y sus pocas interferencias. Además, la sensibilidad del ICP-OES para elementos que forman compuestos refractarios, tales como el I, el P y el S, es mayor.

El ICP-MS es una técnica que se emplea fundamentalmente para su aplicación en el análisis de trazas. Las prestaciones de esta técnica combinan la elevada sensibilidad y limites de detección del horno de grafito con la capacidad multielemental del ICP. Además de que existen pocas interferencias, se pueden compensar fácilmente usando masas alternativas o técnicas de corrección matemáticas. La primera instrumentación en

ICP-MS ha mostrado, no obstante, que existen más interferencias de matriz que las producidas en ICP-OES. Sin embargo, estas interferencias, junto con el alto coste instrumental, están siendo reducidas conforme se avanza en el desarrollo de la técnica.

La elección de la técnica analítica entre las mencionadas no es tarea fácil. Si una aplicación requiere análisis traza de un solo elemento, para un número relativamente pequeño de muestras, o si el coste instrumental es el factor más importante, la Espectroscopia de Absorción Atómica por Llama es, quizás, la técnica más adecuada. Si una aplicación requiere limites de detección muy bajos para unos pocos elementos, la Espectroscopía de Absorción Atómica por Atomización Electrotérmica sería la técnica más recomendable. Si una aplicación requiere bajos limites de detección para un gran número de elementos, la Espectrometría de Masas sería la técnica más adecuada. Cuando se requiere un análisis multielemental de muestras complejas, y alta velocidad de análisis con moderada sensibilidad, la Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente sería la mejor elección. Como es evidente, existen más criterios para la selección de la técnica más apropiada, en función de las características de la muestra y de los recursos disponibles en el laboratorio de análisis.

En las siguientes secciones se describe un poco más detalladamente las técnicas espectroscópicas empleadas durante la realización de este trabajo (ETAAS, ICP-OES e ICP-MS).

3.2 – Espectroscopía de Absorción Atómica por Atomización Electrotérmica (ETAAS)

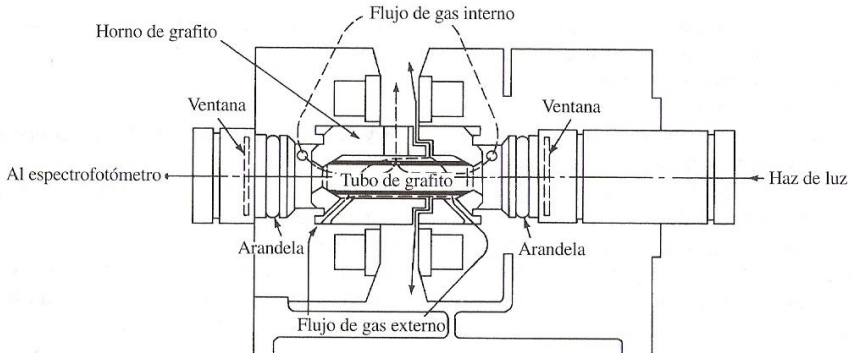
Los atomizadores electrotérmicos, que aparecieron comercialmente en el año 1970, se emplean para realizar medidas de absorción y de fluorescencia atómica pero, por lo general, no han sido empleados para la obtención de espectros de emisión [325]. En este sistema, la atomización tiene lugar en un tubo cilíndrico de grafito, abierto por ambos

extremos, que tiene un orificio central para la introducción de la muestra. En los atomizadores electrotérmicos, unos pocos microlitros de muestra se evaporan primero, a baja temperatura y, luego, se calcinan y atomizan a una temperatura más elevada (entre 2000 y 3000 ºC). Esta técnica proporciona, generalmente, una mayor sensibilidad que la atomización por llama, debido a que toda la muestra se atomiza en un periodo muy corto de tiempo (de unos pocos milisegundos a segundos), y el tiempo de permanencia de los átomos en el camino óptico es más elevado (de un segundo o más). En estas condiciones, la absorción o la fluorescencia de las partículas atomizadas se miden en la zona situada por encima de la superficie calentada. La Figura 3 representa una sección transversal de un atomizador electrotérmico comercial [325].

3.2.1 - Atomizadores electrotérmicos

El tubo de grafito mide unos 5 cm de largo, y tiene un diámetro interno de algo menos de 1 cm. Este tubo es intercambiable y se conecta a un par de contactos eléctricos de grafito que se mantienen dentro de una caja metálica refrigerada por agua, y se ubican en los dos extremos del tubo.

Figura 3. Sección transversal de un horno de grafito.

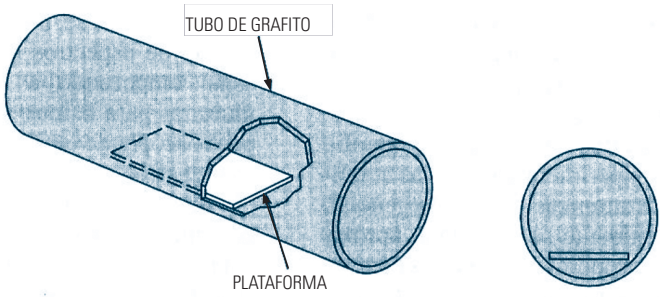


Durante el funcionamiento del instrumento se emplean dos corrientes de gas inerte. La corriente externa evita la entrada de aire exterior y la consiguiente incineración del tubo, y la corriente interna no solo elimina el aire, sino que sirve también para desalojar los vapores procedentes de la matriz de la muestra durante las dos primeras etapas de calentamiento. Esta corriente fluye por entre los dos extremos del tubo y sale por el orificio central del compartimento de muestra.

En la mayor parte de los tubos de grafito, la atomización de la muestra se realiza sobre un soporte denominado "plataforma de L´vov", que se ilustra en la Figura 4 [325]. Esta plataforma, que también es de grafito, se encuentra situada justo debajo del orificio de entrada de la muestra. Mediante la plataforma de L´vov, la muestra se evapora y se calcina de manera habitual, sin embargo, al no estar directamente en contacto con la pared del horno, la atomización de la muestra se retrasa y, de este modo, se obtienen picos más reproducibles.

Durante la atomización de la muestra, parte del analito y de la matriz pueden difundir en el tubo, debido a la porosidad natural del grafito, obteniéndose así señales más bajas del analito. Para evitar este problema se pueden sellar los poros con una delgada capa de carbono pirolítico, haciendo pasar a través del tubo una mezcla de un gas inerte y un hidrocarburo como el metano, mientras se mantiene a una temperatura elevada.

Figura 4. Plataforma de L´vov y su posición en el horno de grafito.



3.2.2 - Señal de salida

En los espectrofotómetros de absorción atómica equipados con un atomizador electrotérmico, los análisis cuantitativos se realizan, generalmente, midiendo la altura de pico, aunque también se puede emplear el área de pico. La señal del detector aumenta al máximo algunos segundos después de la atomización, a la longitud de onda a la que tiene lugar la absorbancia o la fluorescencia, y cae rápidamente a cero cuando los productos de la atomización salen fuera del tubo de grafito. El cambio es tan rápido que se necesita un sistema de adquisición de datos de alta velocidad para poder registrar las señales.

3.2.3 - Características del funcionamiento de los atomizadores electrotérmicos

Los atomizadores electrotérmicos poseen una elevada sensibilidad para pequeños volúmenes de muestra (entre 0,5 y 20 μL). Como desventajas, se puede mencionar que el intervalo analítico es pequeño, siendo, por lo general, menor de dos órdenes de magnitud y que la precisión relativa de los atomizadores electrotérmicos se encuentra, generalmente, en el intervalo del 5 al 10%, en comparación con el 1%, o menos, que se puede esperar de un atomizador con llama o plasma. Otra desventaja es que los métodos de horno son lentos, requiriendo habitualmente varios minutos por elemento, y muestra. Por todo ello, la atomización electrotérmica se aplica normalmente cuando la atomización con llama o plasma proporcionan límites de detección inadecuados [325].

En la mayoría de los atomizadores electrotérmicos las muestras se introducen como disoluciones. Sin embargo, también resulta posible el análisis directo de muestras sólidas, insertando en el horno una navecilla de grafito que contiene la muestra finamente pulverizada. Otra manera es introducir una suspensión de la muestra pulverulenta en el interior del horno de grafito.

3.3 - Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente (ICP-OES)

Un plasma es un estado de la materia que contiene una apreciable fracción (>1%) de electrones e iones positivos en aproximadamente la misma proporción, además de átomos y moléculas neutras [326]. Dos características de los plasmas son que pueden conducir la electricidad, y son afectados por un campo magnético.

Los plasmas eléctricos usados en Espectrometría Atómica analítica son gases ionizados de alta energía. Se generan a partir de gases inertes, aunque también es posible emplear gases reactivos tales como el oxígeno. Las descargas de plasma son considerablemente más calientes que los hornos y las llamas y se usan, por lo tanto, no solo para atomizar la muestra, sino también para excitar e ionizar los átomos con la finalidad de medir emisión iónica y atómica. Entre las fuentes de plasma utilizadas en Espectrometría de Emisión, la de Plasma Acoplado Inductivamente (ICP) es la más eficiente. Otros tipos de plasma también usados son el Plasma de Corriente Directa (DCP) y el Plasma Inducido por Microondas (MIP).

3.3.1 - La fuente de plasma

Para lograr la descarga de plasma acoplado inductivamente, se introduce gas argon (Ar) a través de una antorcha que consiste en tres tubos concéntricos de cuarzo u otro material apropiado. Una bobina de cobre, llamada “bobina de carga” rodea la parte final de la antorcha y se encuentra conectada a un generador de radiofrecuencias (RF). Cuando se aplica energía de radiofrecuencia a la bobina de carga (normalmente de 700 a 1500 W), se produce una corriente alternante que se mueve hacia abajo u oscila a la velocidad correspondiente a la frecuencia del generador (de 27 a 40 MHz). Esta oscilación de RF de la corriente de la bobina causa campos eléctricos y magnéticos en el área de la parte superior de la antorcha.

Cuando el Ar fluye a través de la antorcha, se aplica una chispa al gas, que permite arrancar algunos electrones de los átomos de argon. Estos electrones son entonces atrapados en el campo magnético y son acelerados.

El suministro de energía a los electrones mediante la bobina se conoce como Acoplamiento Inductivo. Estos electrones altamente energéticos colisionan a su vez con otros átomos de argon, arrancando nuevos electrones. Esta ionización colisional del gas argon continua como resultado de una reacción en cadena, convirtiendo el gas inicial en un plasma consistente en electrones, átomos e iones de argon que forman la descarga de ICP. Esta descarga es estabilizada en el interior de la antorcha y la bobina de carga, conforme se aplica energía de RF, a través del proceso de acoplamiento inductivo.

La mayoría de las muestras se introducen como líquidos que se nebulizan formando un aerosol, una fina mezcla de gotas de muestra. El aerosol de muestra es entonces transportado hasta el centro del plasma por el flujo de argon de nebulización. La Figura 5 muestra el esquema de una antorcha usada en ICP.

Figura 5. Esquema de una antorcha de ICP.

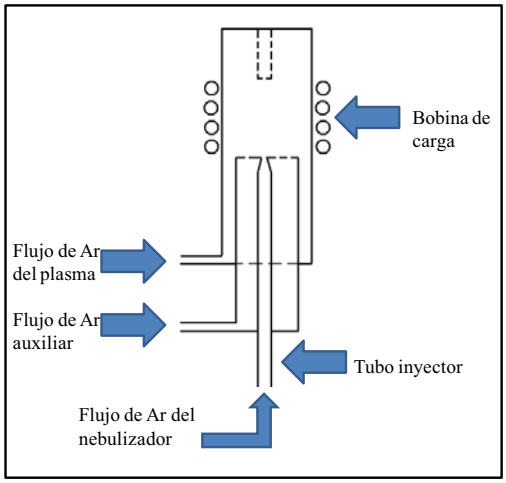
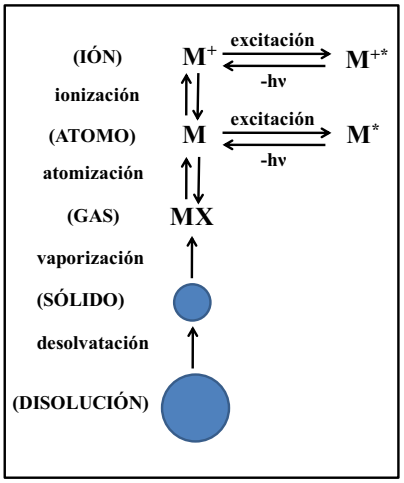


Figura 6. Procesos que tienen lugar en el plasma.



Las funciones del ICP son varias, como se muestra en la secuencia de procesos que ocurren cuando se introduce la muestra (Figura 6). La primera función del plasma es eliminar el disolvente (desolvatación) dando lugar a partículas microscópicas de sal. El siguiente paso implica la descomposición de las partículas de sal, que originan moléculas gaseosas individuales (vaporización). Finalmente, las moléculas son disociadas en átomos libres (atomización). Estos procesos, que ocurren predominantemente en la zona de precalentamiento (ZDP), son los mismos procesos que tienen lugar en llamas y hornos calentados eléctricamente, usados en Absorción Atómica. Una vez que el aerosol ha sido desolvatado, vaporizado y atomizado, pueden ocurrir dos procesos adicionales. Estos procesos son la excitación y la ionización, que suceden principalmente en la zona de radiación inicial (ZRI) y en la zona analítica normal (ZAN), que es la región del plasma utilizada en las medidas de emisión. La Figura 7 muestra las diferentes zonas del plasma.

Las ventajas del ICP sobre otras fuentes utilizadas en medidas de emisión se basan en su capacidad para vaporizar, atomizar, excitar e ionizar de forma eficiente y reproducible un gran número de elementos. Una de las razones más importantes de la superioridad del ICP sobre llamas y hornos estriba en las altas temperaturas presentes en el interior de un plasma. Mientras que las llamas y hornos permiten alcanzar temperaturas máximas alrededor de 3000 K, la temperatura del gas en el centro de la descarga es de alrededor de 6000 K. Estas temperaturas, a parte de mejorar la eficacia de ionización y excitación, permiten reducir o eliminar muchas de las interferencias químicas encontradas en llamas y hornos [327]. La combinación de la alta estabilidad del plasma con la ausencia de interferencias químicas convierte al plasma en una fuente espectroscópica de emisión superior a otras fuentes de descargas eléctricas (arcos, chispas, DCP, MIP). La Figura 8 muestra las temperaturas en las diferentes regiones del plasma.

Figura 7. Zonas del plasma

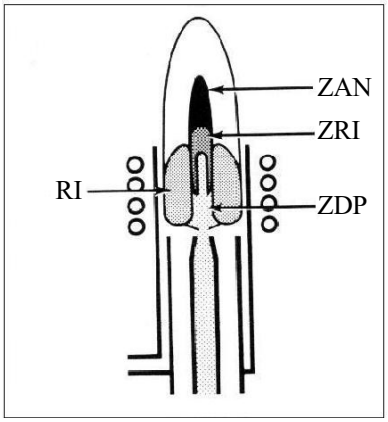
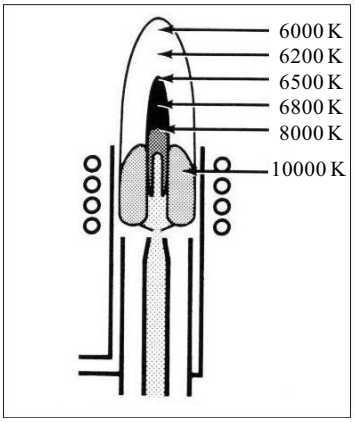


Figura 8. Tª en las regiones del ICP.



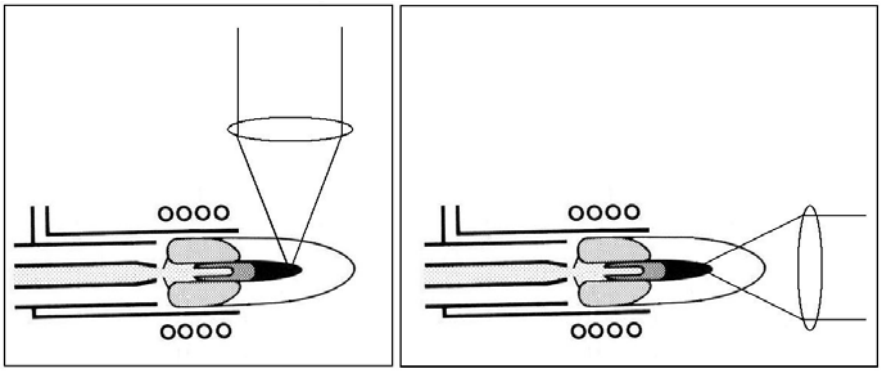
3.3.2 - Detección de la emisión

En ICP-OES, la luz emitida por los átomos e iones excitados en el plasma se mide para obtener información de la composición de la muestra. La mayor parte de los espectrómetros modernos pueden realizar las medidas trabajando con el plasma en modo axial o radial. Cuando se trabaja en modo radial, la radiación emitida por los analitos se mide perpendicularmente a la posición del plasma, mientras que en el modo axial, la radiación emitida por los analitos se mide en la misma dirección en la que se encuentra el plasma (Figura 9).

Según la bibliografía [326], el modo axial proporciona mejores Límites de Detección que el modo radial debido a que los analitos se encuentran más tiempo en la trayectoria del plasma, produciendo una mayor emisión, lo que mejora la sensibilidad de la técnica.

La emisión del plasma es policromática, debido a que las especies excitadas emiten a longitudes de onda diferentes. Esta radiación policromática debe ser separada en longitudes de onda individuales para identificar la emisión de cada especie excitada y medir su intensidad sin interferencia de otras longitudes de onda.

Figura 9. Configuración de la Antorcha



Configuración Radial Configuración Axial

La separación de la luz según la longitud de onda se puede llevar a cabo mediante un monocromador, que permite medir la radiación a una longitud de onda a un tiempo, o un policromador, que permite la medida de diferentes longitudes de onda al mismo tiempo. La detección de la radiación, una vez separadas las diferentes longitudes de onda, se lleva a cabo con un detector fotosensible, como un tubo fotomultiplicador. Los instrumentos más recientes incorporan detectores de fotones multicanal: fotodiodos en serie (PDA), dispositivos de inyección de carga (CID) y dispositivos de acoplamiento de carga (CCD).

3.3.3 - Obtención de la información

La extracción de información cuantitativa y cualitativa de una muestra mediante ICP – OES es sencilla. La información cualitativa implica identificar la presencia de emisión a las longitudes de onda características de los elementos de interés. Las interferencias espectrales que pueden ocurrir debido a la presencia de otras especies en la muestra pueden dar lugar a una incertidumbre en relación a la presencia del elemento en el plasma. Afortunadamente, el número relativamente grande de longitudes de onda alternativas disponibles para medir la emisión de un elemento permite superar dichas interferencias, simplemente cambiando la longitud de onda de la medida.

La información cuantitativa se obtiene relacionando la intensidad de emisión con la concentración en una curva de calibrado. Normalmente tan solo es necesario medir dos o tres patrones, y una disolución del blanco, para calibrar el instrumento, debido a que en ICP-OES las curvas de calibrado son lineales de cuatro a seis órdenes de magnitud.

A parte de poder determinar un gran número de elementos en un amplio rango de concentraciones, la mayor ventaja que presenta el ICP-OES con respecto a hornos y llamas, es que se pueden determinar varios elementos en el mismo análisis. Además, las interferencias químicas son virtualmente eliminadas a causa de la alta temperatura del plasma, y las interferencias físicas pueden ser fácilmente compensadas mediante técnicas que aprovechan la capacidad multielemental del plasma. Las interferencias espectrales presentan las mayores dificultades a la hora de corrección, aunque hoy en día, la disponibilidad de espectrómetros de alta resolución, junto con la flexibilidad de poder elegir entre muchas líneas de emisión, permite un análisis libre de interferencias para muchas muestras [327].

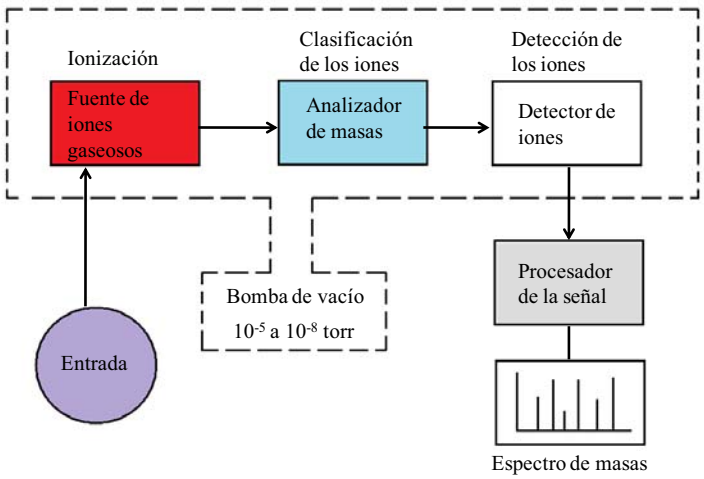
3.4 - Espectrometría de Masas por Plasma Acoplado Inductivamente (ICP-MS)

Un espectrómetro de masas es un instrumento que sirve para separar los iones según su relación masa/carga (m/z). Existen varios tipos de espectrómetros de masas comerciales: espectrómetro de masas cuadrupolar, espectrómetro de masas de tiempo de vuelo y espectrómetro de masas de doble enfoque. La Tabla 9 resume los tipos más importantes de espectrometría de masas atómica e indica que tipo de espectrómetro se utiliza habitualmente en cada tipo de espectrometría de masas atómica [321]. La Figura 10 muestra los componentes principales de cualquier tipo de espectrómetro de masas. El objetivo del sistema de entrada es introducir una pequeña cantidad de muestra en la fuente de iones gaseosos, donde se produce su ionización mediante energía térmica o eléctrica, o mediante el bombardeo con electrones, fotones, iones o moléculas.

Tabla 9. Tipos de espectrometría de masas atómica.

Nombre	Fuente de iones atómicos	Analizador de masas típico
Plasma de acoplamiento inductivo (ICPMS)	Plasma de argón de elevada temperatura	Cuadrupolo
Plasma de corriente continua (DCPMS)	Plasma de argón de elevada temperatura	Cuadrupolo
Plasma inducido por microondas (MIPMS)	Plasma de argón de elevada temperatura	Cuadrupolo
Fuente de chispa (SSMS)	Chispa eléctrica de radiofrecuencia	De doble enfoque
Ionización térmica (TIMS)	Plasma calentado eléctricamente	De doble enfoque
Descarga luminiscente (GDMS)	Plasma de descarga luminiscente	De doble enfoque
Microsonda de laser (LMMS)	Haz laser focalizado	De tiempo de vuelo
Ión secundario (SIMS)	Bombardeo de iones acelerados	De doble enfoque

Figura 10. Componentes de un espectrómetro de masas.



El flujo de iones positivos gaseosos (o iones negativos) es acelerado en el analizador de masas, donde se separan según su relación carga / masa. El número de iones para una relación carga / masa determinada, se mide en el detector de iones, dando información cualitativa y cuantitativa sobre una muestra.

Los espectrómetros de masas requieren un complejo sistema de vacío para mantener una baja presión en todos los componentes, salvo en el sistema de lectura y procesamiento de la señal.

La espectrometría de masas por plasma acoplado inductivamente (ICP-MS) se ha desarrollado desde principios de los años 80, hasta convertirse en una de las técnicas más importantes para el análisis elemental, debido a sus bajos límites de detección, su alto grado de selectividad, y su buena precisión y exactitud [325]. En esta técnica, una antorcha de ICP sirve como atomizador e ionizador de la muestra, que se introduce en forma de disolución, mediante un nebulizador convencional o ultrasónico. También es posible introducir sólidos, empleando técnicas como la ablación por chispa o por laser, o la descarga luminiscente.

En esta técnica, los iones positivos producidos en la antorcha de ICP, se introducen a través de una interfase de vacío diferencial unida a un espectrómetro de masas cuadrupolar. Los espectros producidos de esta manera, consisten en una serie sencilla de picos correspondientes a los isótopos de cada elemento presente, siendo notablemente más sencillos y fáciles de interpretar que los espectros de ICP ópticos convencionales. Esta propiedad es particularmente importante para muestras que contienen elementos como “tierras raras” y otros metales pesados, como el hierro, que producen espectros de emisión ópticos complejos. Estos espectros se utilizan para la determinación cualitativa, semicuantitativa y cuantitativa de los elementos presentes en la muestra, mediante curvas de calibrado en las que se representa el cociente entre el recuento de iones para el

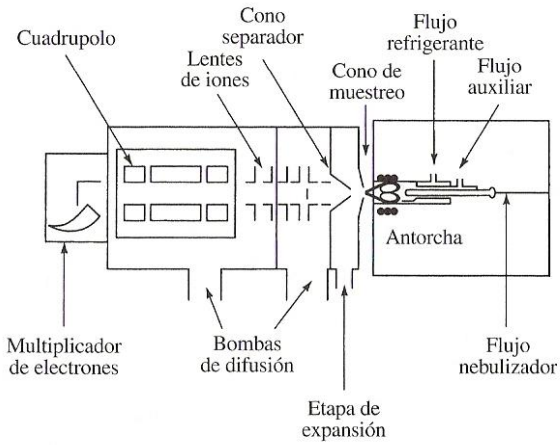
analito respecto del recuento para un patrón interno, en función de la concentración. Los análisis también se pueden realizar mediante la técnica de dilución isotópica.

Uno de los principales atractivos del ICP-MS radica en los límites de detección más bajos con respecto a la detección óptica. Estos, en muchos casos, son iguales e incluso, inferiores a los que se pueden obtener con los métodos de absorción atómica electrotérmica. Otras grandes ventajas del ICP-MS son la capacidad multielemental y la rapidez de los análisis, con tiempos de medida inferiores a 10 segundos, para la mayoría de los elementos. Las desviaciones estándar relativas también son bajas, oscilando entre el 2 y el 4% para concentraciones en las regiones medias de las curvas de calibrado.

3.4.1 - Componentes del ICP-MS

En la Figura 11 [325] se muestra esquemáticamente los componentes de un ICP-MS. El espectrómetro de masas requiere una presión inferior a 10^{-4} torr, mientras que la antorcha de ICP trabaja a presión atmosférica. El acoplamiento de ambos sistemas se realiza mediante un acoplador de interfase de vacío diferencial que consiste en un cono de níquel refrigerado por agua que tiene un pequeño orificio (<1,0 mm) en su centro ("cono de muestreo"). Mediante el empleo de una bomba mecánica, el plasma caliente se transmite a través de este orificio a una región que se mantiene a una presión de 1 torr aproximadamente. En esta región se produce una expansión rápida del gas, que produce su enfriamiento y, a continuación, una fracción del gas pasa a través de un pequeño agujero a un segundo cono denominado "separador" (skimmer) y a una cámara que se encuentra a la presión del espectrómetro de masas. Aquí se aplica un potencial negativo mediante el que los cationes son separados de los electrones y de las especies moleculares, y son acelerados y enfocados hacia el orificio de entrada de un analizador de masas cuadrupolar, mediante una lente magnética de iones [325].

Figura 11. Esquema de un sistema de ICP-MS.



Los espectrómetros de masas atómicos comerciales equipados con una antorcha de ICP permiten determinar alrededor del 90% de los elementos del Sistema Periódico, poseen la capacidad de separar iones que se diferencian en una relación m/z de tan solo una unidad y cuentan con un intervalo dinámico de 6 órdenes de magnitud.

3.4.2 - Interferencias

Las interferencias en espectroscopía de masas atómica pueden ser de dos grandes tipos: interferencias espectroscópicas e interferencias no espectroscópicas. Estas últimas son semejantes a los "efectos matriz" que se producen en los métodos ópticos de absorción y de emisión, mientras que las interferencias espectroscópicas suceden cuando una especie iónica en el plasma tiene los mismos valores m/z que un ión del analito. Dichas interferencias pueden ser de cuatro tipos: iones isobáricos, iones poliatómicos o aductos, iones con doble carga e iones de óxidos refractarios [325].

- **Interferencias isobáricas**

Las especies isobáricas son dos elementos con isótopos que tienen sustancialmente la misma masa. Para un espectrómetro de masas cuadrupolar, las especies isobáricas son isótopos que difieren en su masa en menos de una unidad. Con instrumentos de elevada resolución pueden tolerarse diferencias más pequeñas.

Las correcciones de este problema se pueden llevar a cabo mediante un software adecuado, debido a que los solapamientos isobáricos se predicen exactamente a partir de las tablas de abundancia, y la mayoría de los elementos del Sistema Periódico tienen uno, dos o incluso tres isótopos que están libres de solapamiento isobárico. Algunos instrumentos son capaces de realizar dichas correcciones automáticamente.

- **Interferencias de iones poliatómicos**

Las especies poliatómicas, formadas por interacciones entre las especies del plasma y las especies de la matriz o de la atmosfera, pueden producir interferencias que suelen ser más serias que las causadas por los elementos isobáricos. Como consecuencia pueden formarse diversos iones moleculares que interfieran en las determinaciones. Este tipo de interferencias se encuentra frecuentemente para elementos con valores de m/z por debajo de 82 aproximadamente. Algunas de estas interferencias pueden corregirse con un blanco. En otras, se puede utilizar un isótopo diferente del analito.

- **Interferencia de especies óxido e hidróxido**

El tipo de interferencia más importante en ICP-MS corresponde a los óxidos e hidróxidos del propio analito, de los componentes de la matriz, del disolvente y de los gases del plasma. Estas especies pueden formar iones MO^+ y MOH^+, donde M representa el analito o el elemento de la matriz, cuyos picos pueden superponerse con el pico de uno de los iones del analito.

La reducción de la formación de óxidos e hidróxidos en plasmas es el centro de muchas de las investigaciones actuales. Su formación depende de diversas variables experimentales, como la composición de los gases del plasma, la eliminación del oxigeno, la eficacia de la eliminación del disolvente, la velocidad de flujo del inyector, la potencia de radiofrecuencia o el espaciado del separador del muestreador. En general, se puede actuar sobre todas estas variables para evitar los problemas de solapamiento por óxidos e hidróxidos.

- **Efectos de la matriz**

Los efectos de la matriz son bastante generales, y pueden producirse cuando en la muestra existen elevadas concentraciones de casi cualquier elemento concomitante. Estos efectos suele causar una reducción de la señal del analito, aunque en ciertas condiciones experimentales también se puede observar un aumento de las señales. Por lo general, dichos efectos se pueden minimizar utilizando disoluciones más diluidas, alterando el procedimiento de introducción de la muestra, o separando las especies que lo provocan. Los efectos también pueden ser eliminados mediante la utilización de un patrón interno apropiado.

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OBJETIVOS

OBJETIVOS

El presente trabajo de investigación tiene como objetivo el desarrollo de nuevos métodos de extracción en fase sólida para la preconcentración de metales traza en el agua de mar.

En primer lugar se desarrollará un método consistente en la precomplejación de los metales con 8–Hidroxiquinolina (8-HQ), seguido de la adsorción de los complejos formados en un cartucho comercial de sílice C18 y la elución de los metales adsorbidos con un pequeño volumen de ácido nítrico. Este método será aplicado al estudio de la concentración de diversos metales traza en las aguas de la Ría de Arousa.

En segundo lugar, se sintetizarán diversos polímeros de impronta iónica siguiendo una novedosa metodología de síntesis polimérica denominada "polimerización por precipitación". Para ello, serán empleados diferentes iones metálicos como plantillas (Ni^{2+}, Pb^{2+}), diferentes monómeros (4-vinilpiridina (4-VP), 2-dietilamino etil metacrilato (DEM)) y agentes complejantes (8-hidroxiquinolina (8-HQ), 5-vinil-8-hidroxiquinolina (5-VHQ)).

Los polímeros obtenidos serán caracterizados mediante distintas técnicas como la Fluorescencia de Rayos X (EDXRF), la Microscopía Electrónica de Barrido (SEM) y el Análisis Elemental, y serán evaluados en base a diferentes estudios de adsorción / desorción de los distintos metales de interés (capacidad de carga, volumen de ruptura, exactitud, precisión…). Por último, los polímeros obtenidos serán usados para la determinación de metales traza en muestras de agua de mar y materiales de referencia certificados mediante el empleo de diferentes técnicas analíticas como la Espectroscopía de Absorción Atómica por Atomización Electrotérmica (ETAAS), la Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente (ICP-OES) y la Espectrometría de Masas por Plasma Acoplado Inductivamente (ICP-MS).

RESULTADOS

Evaluation of commercial C18 cartridges for trace elements solid phase extraction from seawater followed by inductively coupled plasma – optical emission spectrometry determination

Jacobo Otero–Romaní, Antonio Moreda–Piñeiro, Adela Bermejo–Barrera,

Pilar Bermejo–Barrera

ANALYTICA CHIMICA ACTA 536 (2005) 213-218

RESUMEN

Durante el siguiente trabajo de investigación se ha desarrollado un método de extracción en fase sólida para la preconcentración de metales traza en el agua de mar. Este método consiste en la precomplejación de los metales disueltos con 8–hidroxiquinolina (8-HQ), seguido de la adsorción de los complejos formados en un cartucho comercial de sílice C18 (Sep-Pack Plus, Waters Co., USA) y la elución de los metales adsorbidos con un pequeño volumen de ácido nítrico para su posterior determinación mediante Espectroscopía de Emisión Óptica por Plasma Acoplado Inductivamente (ICP-OES).

El método propuesto ha sido evaluado con el objetivo de determinar las condiciones óptimas para la extracción simultánea del mayor número posible de metales traza. Para ello, se han estudiado aquellas variables que afectan a la extracción de los complejos metálicos, tales como el pH de la disolución, la velocidad de carga de la muestra y de elución de los analitos, o la concentración y el volumen de la disolución de elución.

El método propuesto permite la determinación simultanea de Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn y Zn, siendo las condiciones óptimas para la extracción: pH igual a 8,0 ± 0,1, velocidad de carga igual a 6,0 ml min^{-1}, velocidad de elución igual a 4,5 ml min^{-1}, volumen de ácido nítrico igual a 2,5 ml y concentración del ácido nítrico 2,0 M.

El método desarrollado presenta bajos límites de detección, y una buena precisión y exactitud, como ha quedado contrastado tras el análisis de diferentes materiales de referencia certificados (CASS-3, SLEW-3 y TM-24). Trabajando con un volumen de muestra de 100 ml se obtuvo un factor de preconcentración de 40, lo suficientemente elevado como para determinar los elementos traza en muestras de agua de mar no contaminadas mediante ICP-OES. Sin embargo, resulta necesaria la realización de una recta de adición estándar para determinar los elementos, debido a la existencia de un cierto efecto matriz, como consecuencia de las sales disueltas en el agua de mar.

El método propuesto permite la preconcentración simultánea de ocho muestras en 30 minutos aproximadamente, mediante el empleo de una bomba peristáltica de 8 vias. Además, aunque los cartuchos de C18 se venden como “de un solo uso”, se ha demostrado que pueden ser reutilizados por lo menos siete veces, sin perder sus propiedades adsorbentes.

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Evaluation of commercial C18 cartridges for trace elements solid phase extraction from seawater followed by inductively coupled plasma – optical emission spectrometry determination

Jacobo Otero–Romaní, Antonio Moreda–Piñeiro, Adela Bermejo–Barrera, Pilar Bermejo–Barrera

Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avda. das Ciencias, s/n. 15782 – Santiago de Compostela. Spain.

Abstract

Possibilities of the use of commercial C18 cartridges to separate and preconcentrate trace elements from seawater have been evaluated. Trace elements (Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn) were previously complexed with 8–hydroxyquinoline, 8–HQ, (5×10^{-4} M as final concentration) at alkaline pH (8.0 ± 0.1) and then they were eluted with 2.5 ml of 2.0 M nitric acid. Metals eluted from cartridges were measured by inductively coupled plasma – optical emission spectrometry (ICP–OES). Variables affecting the metals–8–HQ complexation such as pH and 8–HQ concentration, and affecting the metals–8–HQ complexes solid phase adsorption and elution (load and elution flow rates and concentration and volume of eluting solution) were studied in order to find compromise operating conditions for the simultaneous metals complexation, adsorption and elution. After studies of contamination of commercial C18 cartridges for trace metals, high reagent blanks were reached for Ti so that they are useless for the determination of this element. In addition, commercial C18 cartridges can be used at least seven times without loss of adsorption properties. Working with a seawater sample

volume of 100 ml and using an optimum nitric acid volume of 2.5 ml for elution, a preconcentration factor of 40 was achieved, factor enough high to determine trace elements in unpolluted surface seawater samples by ICP–OES. Analytical performances, such as limits of detection and quantification, repeatability of the over–all procedure and accuracy, by analyzing saline (CASS–3 and SLEW–3) and non saline (TM–24) certified reference materials, were finally assessed.

Keywords

Commercial C18 cartridges, solid phase extraction, trace metals, unpolluted surface seawater, inductively coupled plasma optical emission spectrometry.

1. Introduction

Seawater analysis for heavy metals and other contaminants is increasing in contamination studies owing to the need to guarantee the good quality of seawater for different purposes. This is especially important for coastal or estuarine seawater because of the large number of industries dealing with seafood production which use the estuaries to keep, mainly, molluscs and seaweed. In fact, there is European Directive relating to the quality of water to keep molluscs which limits the levels of several heavy metals such as Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn [1].

Since trace metals concentration levels in seawater are very low [2], sensitive analytical techniques as well as pre–concentration methods must be used to assess background levels of trace elements. In addition, the saline composition of seawater can be an important drawback in seawater analysis because it is a source of high background signals [3] and, when using inductively coupled plasma –

optical emission spectrometry (ICP–OES), a source of spectral [4] and matrix interferences [5]. To overcome these problems, different procedures such as liquid–liquid extraction, coprecipitation, electrodeposition and solid phase extraction, mainly using ionic exchange resins, have been developed. Among all these procedures, solid phase extraction techniques are quite popular since they offer a number of important advantages over other preconcentration methods. One of these benefits is that sorbent material can be packed into mini–columns or cartridges which provide sufficient exchanged / adsorption capacity for many applications [6,7].

The adsorption of non polar species onto active carbon, silica gel or alumina is well–established for non polar and less polar organic analytes. However, a previous derivatisation stage is mandatory for inorganic species because only uncharged (non polar) species can interact with the adsorbent material. Therefore, these methods involve a previous complexation of trace metals with an organic chelating reagent [8,9,10,11,12], which must form uncharged metal–complexes. 8–hydroxyquinoline (8–HQ) forms uncharged chelates with at least 60 elements [13,14] and offers as advantage its lack of affinity for alkaline and alkaline earth metals. Some works dealing with the adsorption of metals–oxinates, such as the applications by Abbasse et al [7,15] and Skinner and Salin [16], can be found in literature and all these procedures use high purity C18 adsorbent material laboratory–packed into columns.

One of the aims of the current work is evaluating the possibilities of use of commercial C18 cartridges, commonly used for non polar organic compounds pre–concentration and for clean–up proposes, for trace metals solid phase extraction. This evaluation has implied a study on the trace metals released from new

commercial C18 cartridges, and the findings of a procedure to wash and prepare the cartridges for trace elements. In addition, the possibility of reusing the C18 cartridges was also considered in this study.

2. Experimental

2.1. Instrumentation

An Optima 3300 DV inductively coupled plasma optical emission spectrometer (Perkin Elmer, Norwalk, USA) equipped with an autosampler AS 91 (Perkin Elmer) and a GemCone cross flow nebulizer type (Perkin Elmer) and a cyclonic spray chamber (Perkin Elmer) was used for all measurements. An 8–ways Gilson peristaltic pump (Gilson, Villiers, France) equipped with 3.18 mm i. d. Tygon tubes (Gilson) was used to drive samples through the C18 chemically bonded silica gel (Sep–Pack Plus – 360 mg, Waters, Milford, MA, USA). A Class–100 clean fume hood (Telstar S.A., Terrassa, Spain) was used to perform all experiments in order to avoid contamination of samples and reagents.

2.2. Reagents

Ultra–pure water of resistivity 18 MΩ cm obtained from a Milli–Q purification device (Millipore Co., Bedford, MA, USA). High purity nitric acid and hydrochloric acid were from Panreac (Barcelona, Spain). High purity ammonia and analytical grade methanol were purchased from Merck (Darmstad, Germany). 8-Hydroxyquinoline solution (0.5 M) was prepared from analytical grade reagent (Merck) by dissolving in a 2.0 M hydrochloric acid solution. Ammonium acetate (0.01 M) was prepared from analytical grade reagent ammonium acetate (Merck) by dissolving in Milli–Q water. Multi–elemental standard solution containing As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, Ti and Zn at 100 μg ml^{-1} was obtained from Perkin

Elmer. Single standard solutions of As, Cd, Cr, Ni, Pb, V, Sn and Zn (Merck), Al, Cu and Fe (Perkin Elmer) and Mn (Panreac) at 1000 μg ml^{-1} were also used. Coastal seawater (CASS–3) and estuarine seawater (SLEW–3) certified reference materials were obtained from the National Research Council of Canada. Lake water (TM–24) certified reference material was purchased by the National Water Research Institute of Canada.

All glass– and plastic–ware were rigorously cleaned and kept into 10 %(m/m) nitric acid for at least 48 h. The material was then rinsed three times with Milli–Q water.

2.3. Seawater collection

Seawater samples were collected from the Ría de Muros–Noia estuary (North–west Spain) in pre–cleaned high density polyethylene bottles. After collection, seawater samples were acidified at pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption onto the inner bottles walls. Acidified seawater samples were then filtered through a 0.45 μm polycarbonate membrane Nucleopore filter (Millipore).

2.4. C18 cartridge preparation

C18 sorbent packing material was firstly washed by passing 25 ml of 2.0 M nitric acid solution at a flow rate of 1.5 ml min^{-1} in order to remove trace metals adsorbed in the packing material. Then, the C18 cartridges were rinsed by pumping 25 ml of Milli–Q water and they were activated by washing with 10 ml of methanol and rinsed with 10 ml of Milli–Q water. Finally, the activated silica into cartridges was conditioned by passing 10 ml of 0.01 M ammonium acetate (pH 7.0).

2.5. C18 cartridge solid phase extraction procedure

A volume of 0.1 ml of 0.5 M 8-HQ solution was added to 100 ml of acidified seawater samples, giving a final 8-HQ concentration of 5×10^{-4} M. Then, the pH was readjusted to 8.0 ± 0.1 by addition of a small volume (1–2 ml) of a 5.0 M ammonia solution. The seawater sample was then passed through a cleaned and conditioned C18 cartridge at a fixed flow rate of 6.0 ml min^{-1} by using a peristaltic pump. The cartridges were then rinsed with 10 ml of 0.01 M ammonium acetate in order to remove the saline matrix, and the adsorbed metal–complexes were eluted with 2.5 ml of 2.0 M nitric acid at a flow rate of 4.5 ml min^{-1}. A pre–concentration factor of 40 was achieved under these operating conditions.

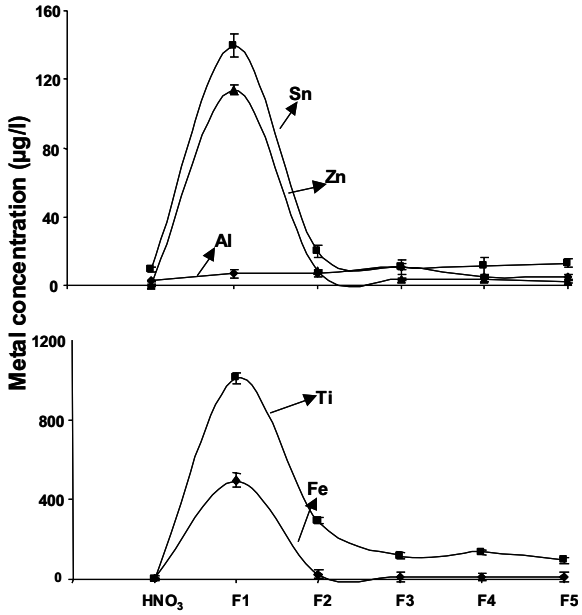
2.6. ICP–OES measurements

Eluted trace elements were measured by ICP–OES (axial configuration, radiofrequency power of 1300 W and plasma, auxiliary and nebulizer gas flows of 15.0, 0.5 and 0.8 l min^{-1}, respectively) without dilution. The standard addition method, covering analyte concentrations of 0, 0.04, 0.08 and 0.16 mg l^{-1} (Cd, Cu, Ni, Pb and V); 0, 0.2, 0.4 and 0.8 mg l^{-1} (Mn and Zn); 0, 0.4, 0.8 and 1.6 mg l^{-1} (Sn); 0, 1.0, 2.0 and 4.0 mg l^{-1} (Fe) and 0, 2.0, 4.0 and 8.0 mg l^{-1} (Al), taking into account a pre–concentration factor of 40, was used.

3. Results and discussion

In order to verify possible trace metal contamination of commercial C18 cartridges, five different aliquots (5 ml each one) of 2.0 M nitric acid solution were passed through the cartridges at a flow rate of 1.5 ml min^{-1}. The five sequential eluates, as well as 5 ml of a 2.0 M nitric acid solution (blank), were analysed by ICP–OES.

Figure 1. Concentrations of selected metals in 2.0 M nitric acid washing solutions after passing through commercial Sep–Pack Plus C18 cartridges.



Negligible concentrations in the five washing solutions were found for As, Cd, Cr, Cu, Mn, Ni and Pb. However, contamination was observed for Al, Fe, Ti, Sn and Zn, mainly after passing the first 5 ml nitric acid aliquot (Figure 1). These metals concentrations were reduced moreover the volume of nitric acid solution was increased and after passing 25 ml of the washing solution (the last 5 ml aliquot) the concentration of most of them were similar than those metal concentrations in 2.0 M nitric acid solution (blank). However, Ti concentrations found in the last washing solution was still extremely high, around 97 µg l^{-1}. Contamination of C18 cartridges by Ti must be probably due to the use of Ti–based devices for grinding and packing the adsorbent material. Further experiments, implying a more concentrated nitric acid solution as well as larger volumes of washing solution,

were carried out in order to reduce the levels of Ti before using the cartridges for trace elements pre–concentration. All these experiments have resulted in high levels of Ti released from C18 cartridges. Therefore, Ti was not considered in further studies because of high Ti levels released from commercial C18 cartridges.

3.1. C18 cartridges solid phase extraction

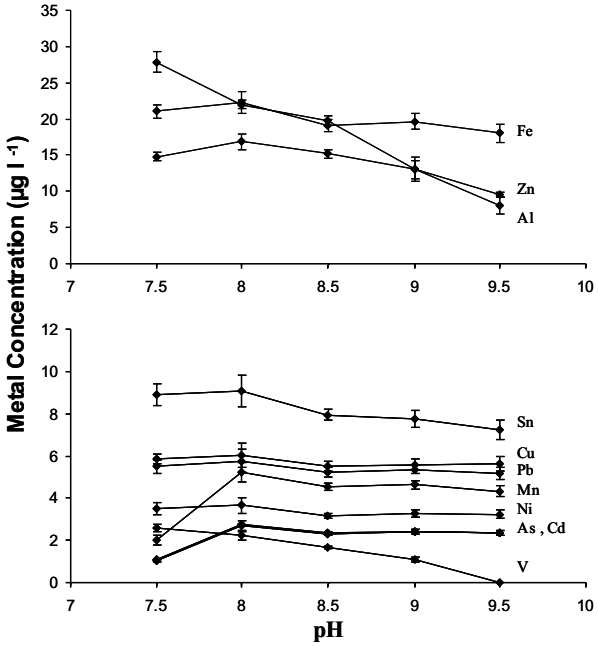
Preliminary studies on the effect of the 8–HQ concentration on the formation of metal–8–HQ complexes have revealed that this variable is not significant. Therefore, an excess of 8–HQ concentration of 5×10^{-4} M to guarantee the formation of neutral metal–oxinates [14] was used for all experiments. This 8–HQ concentration is quite similar than those previously reported by other authors [7, 16].

3.1.1. Effect of the pH on the metal–complexes formation

The efficiency of 8–HQ to react with metals and form uncharged metal–8–HQ complexes is largely dependent on pH. This is because 8–HQ is an ampholyte, forming oxinium (8–hydroxyquinolinium) ion by protonation of N in acid solutions and oxinate ion in basic solutions [13]. Most of the divalent and trivalent elements react with the oxinate form and the establishment of optimum pH values for the different elements is necessary.

To study the effect of pH on the efficiency of metal–8–HQ complexes formation, different aliquots of 100 ml from an acidified seawater sample were spiked with 3 $\mu g\ l^{-1}$ of each metal. Trace metals were complexed with 8–HQ and the pH was readjusted by adding small volumes of 5.0 M ammonia solution to obtain pH values within the 7.5 – 9.5 range. Each pH value was tested three times. Solutions were passed through the pre–conditioned commercial C18 cartridges at a fixed load flow rate of 1.5 ml min^{-1} by using a peristaltic pump.

Figure 2. Effect of the pH on the metal complexation with 8–HQ.



The cartridges were then rinsed with 10 ml of 0.01 M ammonium acetate in order to remove the saline matrix, and the adsorbed metal–8–HQ were eluted with 5.0 ml of 2.0 M nitric acid at an elution flow rate of 0.6 ml min^{-1}. Trace metal concentrations for each experiment at each pH value after analyzing the spiked seawater samples using metal standards in 2.0 M nitric acid for calibration are plotted in Figure 2.

It can be seen that most of the studied metals are largely formed/retained at pH 8.0. Elements such as Cu, Ni, Fe, Sn and Pb appear not being dependent on the pH value, while As, Cd and Mn are efficiently complexed and retained at pHs within 8.0 – 9.5 range, decreasing at pHs lower than 8.0. However, V and Al are not complexed and/or retained at high pH values, and a decrease on the

formation/adsorption efficiency is observed for pH higher than 7.5. This result can be expected for V, because the most stable species is VO^{2+}, which is only complexed by 8–HQ in acid medium (pH 7.0 or lower than 7). At alkaline pH, VO^{2+} is oxidised to VO_4^{3-}, species which is not complexed by the oxinate form of 8–HQ [7]. Finally, Zn offers a particular trend, showing decreases on the solid phase extraction efficiency when using pH higher and lower than 8.0. Therefore and in order to fix compromise conditions for all elements tested, a pH of 8.0 was chosen, pH that results adequate for most of the elements studied, except for Al and V, for which a pH close to 7 would be the best.

3.1.2. Effect of volume and concentration of nitric acid for elution

Elution conditions were carefully studied. Since uncharged metal–8–HQ complexes are adsorbed onto the adsorbent packing material in C18 cartridges, elution of metals must imply the metal–8–HQ destruction, obtaining divalent and trivalent charged metals which do not interact with the adsorbent. This is easily reached using an acid [7, 15] as eluting solution or using methanol [16]. Therefore, different nitric acid solutions were tested in order to elute metals. Two variables affecting metals elution were studied: the nitric acid concentration and the nitric acid volume. This last variable should adopt the lowest value so that a large pre–concentration factor could be assessed. Therefore, two nitric acid solutions at 2.0 and 4.0 M were simultaneously studied for eluting volumes between 2.5 and 6.0 ml (pre–concentration factors within the 40.0 – 16.7 range). The eluting volume of 2.5 ml was the lowest volume studied because the ICP–OES used requires at least 2.0 ml to perform the measurements. It must be also noticed that there are many systems that can work with smaller volumes, hence higher preconcentration factor may be possible.

Under optimum values for pH, metal–8–HQ retained on the C18 cartridge were eluted at a fixed flow rate of 0.6 ml min^{-1} using different nitric acid volumes and nitric acid concentrations. Results have shown that efficient metals elution are reached under all nitric acid volumes when using 2.0 M nitric acid as eluting solution Similar results have been obtained when using 4.0 M nitric acid. Therefore, the lowest nitric acid concentration (2.0 M) and the lowest nitric acid volume (2.5 ml) were chosen, implying a pre–concentration factor of 40.

3.1.3. Effect of the load flow rate on the metal–complexes solid phase extraction

Load flow rate through the C18 cartridges should be fast enough to perform the solid phase extraction in a short time, but it should be slow enough to allow the interaction between the metal–complexes and the adsorbent packing material in the cartridges.

To study this variable, different aliquots of 100 ml from an acidified seawater sample spiked with 3 µg l^{-1} of each metal were 8–HQ complexed at a pH 8.0 and the solutions were loaded on the C18 cartridges at different flow rates, from 1.5 ml min^{-1} to 9.0 ml min^{-1}. After sample load, the cartridges were rinsed with 0.01 M ammonium acetate solution (10 ml) and the retained metal–8–HQ were eluted with 2.5 ml of a 2.0 M nitric acid at a flow rate of 0.6 ml min^{-1}. Results have shown that 8–HQ can be retained when pumping solutions at flow rates within the 1.5 and 6.0 ml min^{-1}. Larger load flow rates than 6.0 ml min^{-1} has led to uncompleted adsorption for some elements, such as Mn. Therefore, a sample load flow rate of 6.0 ml min^{-1} was selected. This rate is high enough to load the sample in a moderate short time and so that metal–8–HQ can interact with the adsorbent material.

3.1.4. Effect of the elution flow rate

The effect of elution flow rate was finally studied. After fixing all variables at optimum values (pH 8.0, load flow rate 6.0 ml min^{-1} and 2.5 ml of 2.0 M nitric acid for elution) metal–8–HQ retained were eluted at flow rates from 0.6 ml min^{-1} to 4.5 ml min^{-1}. Results showed that metals are efficiently eluted from cartridges at all elution flow rates studied and an elution flow rate of 4.5 min^{-1} was selected.

3.1.5. Study of the number of solid phase extraction performed with conventional C18 cartridges

Commercial C18 cartridges, although sold as single use cartridges, were tested in order to know the number of sequential solid phase extraction that can be performed without losses on metals–8–HQ adsorption and / or metals elution. This experiment was carried out with three different cartridges which were used several times for a period of 2 months. Different seawater samples spiked with variable concentrations of metals were determined and the analytical recoveries for each element obtained. Results have shown that some elements such as Cu, Fe, Pb, Sn and Zn are not efficiently retained / elute after seven uses, and analytical recovery is poor when using the same C18 cartridges more than seven times. In addition, it has been found that for some elements, even being efficiently retained after seven uses, the repeatability was got worse when using the cartridges more than seven times. Therefore, conventional C18 cartridges can be used at least seven times without losing of efficiency of the adsorbent packing material adsorption properties.

3.2. Analytical performance

Although solid phase extraction implies metals separation from the seawater matrix, a comparison between calibration matched with 2.0 M nitric acid and

standard addition was established for each element in order to study possible matrix effect. The standard addition technique was obtained after spiking different 100 ml aliquots from a same acidified seawater sample by triplicate with different metal concentrations (up to 4.0 µg l^{-1} for As, Cd, Cu, Ni, Pb and V, 20 µg l^{-1} for Mn and Zn, 40 µg l^{-1} for Sn, 100 µg l^{-1} for Fe and 200 µg l^{-1} for Al). After solid phase extraction, the eluates were measured by ICP–OES (section 2.6.) and 2.0 M nitric acid calibrations and standard addition graphs were obtained. The standard deviation and the mean for the slopes of two standard addition graphs and six external 2.0 M nitric acid calibrations were statistically compared by using the Cochran'C and Bartlett's tests at a 95.0 % to compare variances, and the ANOVA test to compare means. It can be said that slopes for external 2.0 M nitric acid calibration and standard addition graphs are statistically different for all cases, except for V. Therefore, although a large volume of ammonium acetate solution was used to remove possible salts on C18 cartridges just before metals elution, a certain matrix effect was observed when determining all elements, being necessary the establishment of a standard addition graph to perform seawater analysis.

The limit of detection, given by $LOD=3*(Sd/m)$, where Sd is the standard deviation of eleven measurements of a reagent blank, and m is the slope of the standard addition graph, were calculated. After a pre–concentration factor of 40, LODs of 1.28, 0.04, 0.04, 0.02, 0.19, 0.001, 0.03, 0.03, 0.07, 0.06 and 1.13 µg l^{-1}, were reached for Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn, respectively. Such LODs are low enough to determine trace metals levels in unpolluted seawater samples.

The repeatability of the over–all procedure (solid phase extraction and ICP–OES determination) was assessed by analyzing three times a same seawater sample. The

average percent relative standard deviations (RSDs) were 3.5, 0.7, 3.7, 6.3, 4.7, 5.3, 0.2, 5.2, 5.4, 7.5 and 3.5 % for Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn, respectively. Since these results it can be concluded that the procedure offer good repeatability for all elements (RSDs lower than 10%).

The accuracy of the method was verified by studying the analytical recovery and by analyzing different saline water certified reference materials (CASS–3 and SLEW–3) and a non saline water certified reference material (TM–24). Analytical recovery was assessed for three concentration levels, after spiking two different seawater samples with analyte concentrations of 1.0, 2.0 and 4.0 μg l^{-1} for As, Cd, Cu, Ni, Pb and V; 5, 10 and 20 μg l^{-1} for Mn and Zn; 10, 20 and 40 μg l^{-1} for Sn; 25, 50 and 100 μg l^{-1} for Fe and 50, 100 and 200 μg l^{-1} for Al. The concentration levels tried are representative of clean surface seawaters. Each concentration level in each seawater samples was tried three times, thus, analytical recoveries listed in Table 1 are the mean of six independent measurements. From Table 1 it can be concluded that good analytical recovery (within the 90 – 110 % range) are reached for all elements at all concentration levels, except for V at the lowest concentration level. This could be attributed to a less efficient solid phase extraction for V for which a more acidic pH (lower than 8.0) would be desirable.

CASS–3, SLEW–3 and TM–24 certified reference materials were analyzed by triplicate for most of the elements. Determinations were performed by using the standard addition technique. Results, listed in Table 2, reveal good agreement between found concentrations and certified values for most of the elements in the three certified reference materials. However, Cd, Pb and Zn have not been determined in CASS–3 and SLEW–3 because certified concentrations of these elements in these materials are lower than the LODs of the method for these elements.

Table 1. Percent analytical recoveries (n = 6) of the method.

	Percent analytical recoveries		
	Low [a]	Medium [b]	High [c]
Al	87.3± 3.0	92.0 ± 1.3	101.2 ± 1.7
As	102.3 ± 1.5	101.6 ± 0.2	100.1± 0.1
Cd	103.3 ± 0.8	101.7± 0.4	100.3 ± 0.1
Cu	107.6± 1.7	101.9 ± 3.2	100.9 ± 0.5
Fe	95.1± 2.3	98.7± 1.9	99.7 ± 0.3
Mn	104.9± 4.0	102.1± 0.9	100.3 ± 0.3
Ni	106.3± 1.3	104.7 ± 2.7	100.8 ± 0.8
Pb	98.3± 1.8	99.0 ± 5.2	99.9 ± 0.1
Sn	92.2± 0.3	92.5± 5.3	99.8 ± 0.3
V	70.7± 5.2	97.9 ± 0.6	87.7 ± 6.6
Zn	108.2± 0.4	102.7± 3.4	100.2 ± 0.7

(a) 1 μg l^{-1} for As, Cd, Cu, Ni, Pb and V; 5 μg l^{-1} for Mn and Zn; 10 μg l^{-1} for Sn; 25 μg l^{-1} for Fe and 50 μg l^{-1} for Al. (b) 2 μg l^{-1} for As, Cd, Cu, Ni, Pb and V; 10 μg l^{-1} for Mn and Zn; 20 μg l^{-1} for Sn; 50 μg l^{-1} for Fe and 100 μg l^{-1} for Al. (c) 4 μg l^{-1} for As, Cd, Cu, Ni, Pb and V; 20 μg l^{-1} for Mn and Zn; 40 μg l^{-1} for Sn; 100 μg l^{-1} for Fe and 200 μg l^{-1} for Al

In addition, V concentration found is not within the V certified concentration range due to a pH of 8.0 is not the optimum to form V–8–HQ complexes (see Figure 2). Finally and although As levels in the three certified reference materials are quite higher than the LOD of the method for this element, As in the three certified reference materials was not determined. Arsenic is mainly as $HAsO_4^{2-}$ (As(V)) in seawater [2] but 8–HQ only reacts with As(III) species. Therefore, good analytical recovery is reached when spiking with As(III), as shown in Table 1, but lack of accuracy has been obtained after analyzing certified reference materials, in which As occurs as As(V).

Table 2. Analysis of certified reference materials (n = 3).

	SLEW–3		CASS–3		TM–24	
	Certified / µg l^{-1}	Found / µg l^{-1}	Certified / µg l^{-1}	Found / µg l^{-1}	Certified / µg l^{-1}	Found / µg l^{-1}
Al	-	-	-	-	22 ± 19	28 ± 1
As	1.36 ± 0.09	(a)	1.09 ± 0.07	(a)	2.7 ± 1.0	(a)
Cd	0.048 ± 0.004	< LOD	0.030 ± 0.005	< LOD	12.5 ± 3.5	14.6 ± 1.0
Cu	1.55 ± 0.12	1.96 ± 0.12	0.517 ± 0.062	0.648 ± 0.060	8.0 ± 4.1	9.0 ± 0.3
Fe	0.568 ± 0.059	0.520 ± 0.002	1.26 ± 0.17	1.09 ± 0.12	5.4 ± 2.3	5.4 ± 0.5
Mn	1.61 ± 0.22	1.62 ± 0.19	2.51 ± 0.36	2.78 ± 0.38	3.1 ± 1.4	2.9 ± 0.1
Ni	1.23 ± 0.07	1.35 ± 0.13	0.386 ± 0.062	0.353 ± 0.033	3.5 ± 3.0	3.7 ± 0.1
Pb	0.0090 ± 0.0014	< LOD	0.012 ± 0.004	< LOD	7.3 ± 2.6	9.7 ± 0.2
V	2.57 ± 0.31	1.96± 0.16	-	-	6.7 ± 2.0	4.0 ± 1.0
Zn	0.201 ± 0.037	< LOD	1.24 ± 0.25	< LOD	13.6 ± 4.8	20.8 ± 0.4

(a) Not determined

4. Conclusions

The use of commercial Sep–Pack Plus C18 silica cartridges, commonly used for non polar organic analytes, can be used to pre–concentrate trace elements from seawater after 8–HQ complexation. A previous washing step, involving the use of 25 ml of 2.0 M nitric acid, is required to avoid contamination from the silica adsorbent packing material. After this wash treatment, contamination problems are significant for Ti and this element can not be pre–concentrated because of high reagent blanks. In addition, although C18 cartridges are sold as single use cartridges, they can be reused at least seven times without losing of analytical performances. Compromise conditions for different variables affecting the metal–8–HQ formation and the adsorption on and elution from the adsorbent silica allow

the simultaneous solid phase extraction of Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn from seawater. The optimized solid phase extraction is fast and it offers a high sample treatment rate (simultaneous solid phase extraction of eight samples can be carried out using an 8–ways peristaltic pump in 30 min). In addition, the sample volume required is low when comparing to other proposed methods [7,16], which imply a fast sampling and advantages when storing samples. This fact combined with the adequate sensitivity by ICP–OES of new generation allows the application of this method to routine multi–element analysis of seawater.

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Synthesis, characterization and evaluation of ionic-imprinted polymers for solid-phase extraction of nickel from seawater

Jacobo Otero-Romaní, Antonio Moreda-Piñeiro, Pilar Bermejo-Barrera, Antonio Martin-Esteban

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RESUMEN

En el siguiente trabajo de investigación se han sintetizado diversos polímeros de impronta iónica para su utilización como fases sólidas en procedimientos de extracción y preconcentración de niquel (II) en muestras de agua de mar. Para ello se ha empleado un proceso de "polimerización por precipitación", utilizando como plantilla níquel (II), o un complejo de níquel (II)-8-HQ, y como monómero la 4-vinilpiridina (4-VP) o el 2-dietilamino etil metacrilato (DEM). En todos los casos se ha empleado 2,2'-azobisisobutironitrilo (AIBN) como iniciador, divinilbenceno (DVB) como entrecruzante, y una mezcla de acetonitrilo / tolueno (3:1) como porogen.

Las partículas obtenidas fueron sometidas a un proceso de limpieza para eliminar el analito plantilla de las cavidades formadas, y fueron caracterizadas mediante EDXRF, SEM, y Análisis Elemental. Los mejores resultados, en cuanto al reconocimiento del analito plantilla, fueron obtenidos con aquellos polímeros sintetizados en presencia del agente complejante (8-HQ) y del DEM, siendo posteriormente evaluados con el objetivo de determinar los parámetros óptimos que permitiesen la recuperación cuantitativa del níquel en el agua de mar. Estos parámetros fueron: pH igual a 8,5 ± 0,5, volumen de elución igual a 2,5 mL de ácido nítrico 2,0 M, velocidad de carga igual a 10 ml min^{-1} y velocidad de elución inferior a 1,5 ml $min^{-1.}$ El compuesto sintetizado permite cargar 250 ml de muestra a través de un cartucho que contenga 300 mg de polímero, sin que se alcance el volumen de ruptura. Por lo tanto, cuando la elución se lleva a cabo con 2,5 ml de ácido nítrico se obtiene un factor de preconcentración de 100, lo que permite alcanzar un límite de detección de 0,050 μg l^{-1} cuando se utiliza la ETAAS como técnica analítica de detección.

El método propuesto presenta una buena precisión (6%) y exactitud, lo cual ha quedado contrastado mediante la realización de sendos estudios de reproducibilidad (n=11) y recuperación analítica, y mediante el análisis de diferentes materiales de referencia certificados (SLEW-3, TM-23.3 y TM-24). Por otro lado, el polímero sintetizado no retiene los elementos mayoritarios presentes en el agua de mar (sodio, potasio, calcio, magnesio), ofreciendo la posibilidad de eliminar eficazmente la matriz salina de las muestras. Este hecho se ha demostrado mediante la comparación estadística de tres rectas de calibrado y tres rectas de adición estándar. Por lo tanto, después de la preconcentración de una muestra de agua de mar en este polímero resulta posible la determinación directa del níquel en los eluatos mediante la realización de una simple recta de calibrado, no siendo necesario el uso de una recta de adición estándar.

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Synthesis, characterization and evaluation of ionic-imprinted polymers for solid-phase extraction of nickel from seawater

Jacobo Otero-Romaní[1], Antonio Moreda-Piñeiro[1], Pilar Bermejo-Barrera[1], Antonio Martin-Esteban[2]

(1) Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain. (2) Department of Environment. National Institute for Agriculture and Food Research (INIA). Carretera de A Coruña, Km 7,5. 28040 – Madrid. Spain.

Abstract

Several nickel ion imprinted polymers were prepared via precipitation polymerization using 4-vinylpyridine or 2-(diethylamino) ethyl methacrylate as monomers (vinylated reagents) and a crosslinking agent divinylbenzene in the presence of nickel(II) alone or nickel(II) and 8-hydroxyquinoline (non-vinylated reagent). For all cases, 2,2′-azobisisobutyronitrile (AIBN) was used as an initiator and an acetonitrile/toluene (3:1) mixture was chosen as a porogen. After packing the polymer particles into empty SPE cartridges, nickel(II) ions were removed by washing with 50 mL of 2.0 M nitric acid. Characterization of the polymer particles has been carried out by scanning electron microscopy, energy dispersive X ray fluorescence and elemental analysis. The best nickel imprinting properties were given by polymers synthesized in the presence of 8-hydroxyquinoline and 2-(diethylamino) ethyl methacrylate as a monomer. The optimum pH for quantitative nickel retention was 8.5 ± 0.5, while elution was completed with 2.5 mL of 2.0 M nitric acid. When using polymer masses of 300 mg, sample volumes until 250 mL can be passed through the cartridges without reaching the breakthrough

volume. Therefore, a pre-concentration factor of 100 has been reached when eluting with 2.5 mL of the elution solution. Electrothermal atomic absorption spectrometry has been used as a detector for nickel determination. The limit of detection of the method was 0.050 μg l^{-1} (pre-concentration factor of 100), while the relative standard deviation for eleven replicates was 6%. Accuracy of the method was assessed by analyzing different certified reference materials: SLEW-3 (estuarine water) and TM-23.3 and TM-24 (lake water).

Keywords

Ion imprinted polymer, nickel, seawater, solid phase extraction, electrothermal atomic absorption spectrometry

Introduction

Electrothermal atomic absorption spectrometry (ETAAS) and multi-element detectors such as inductively coupled plasma – optical emission spectrometry / mass spectrometry (ICP-OES/MS) have been commonly used to assess trace elements in waters. However, lack of sensitivity and accuracy can be found when using these techniques for seawater analysis. As recently reviewed by Rao *et al*. [1] ETAAS combining the multiple injection technique and the Zeeman correction has been used for direct determination of Ni in seawater [2,3]. Although charring temperatures till 1700°C can be used without nickel losses [2], the seawater matrix (mainly NaCl) has been reported to be held in the graphite tube even at temperatures of 1700°C [4]. This fact generates high background signals, scattering, and a strong matrix effect in direct ETAAS determinations that lead to a worsening on sensitivity and even loss of accuracy [3]. In addition, spectral and

matrix interferences have been also reported for ICP-OES measurements [5,6], and the removal of the salt matrix prior seawater analysis is highly recommended [7].

Therefore, separation/pre-concentration methods are commonly used as previous stages for trace element determinations in seawater samples. Solid-phase extraction (SPE), mainly using ionic exchange resins [8], functionalized chelating resins [9] or high purity C_{18} adsorbent material [7,10,11] are mostly used to pre-concentrate trace elements and to remove the salt matrix. SPE has become quite popular since it offers many practical and operating advantages over other pre-concentration methods [8]. However, interfering compounds might be coextracted with the target analytes on conventional sorbents. To overcome this lack of selectivity, the use of molecularly imprinted polymers (MIPs) have been proposed, especially suited for the clean-up of organic compounds [12]. The synthesis of MIPs is fast and quite cheap and the material provides a high degree of molecular recognition. As reported, molecular imprinting polymerization is based on the preparation of a highly cross-linked polymer around a template in the presence of a suitable monomer [12]. At present, the imprinting of organic molecules is a well-established technology; however, few attempts have been made for ions, such as trace elements [13,14]. In such cases, the synthesized sorbent is called an ionic imprinted polymer (IIP). IIPs have been mostly synthesized for recognizing lanthanides [15,16,17,18,19], actinides [20,21] and noble metals [22]. Recently, a variety of IIPs have been prepared as selective sorbents for the SPE of heavy metals and transition elements such as copper [23,24], cadmium [25,26], cobalt [27] , selenium [28] and nickel [27,29,30].

Most of the IIPs have been obtained by bulk polymerization, and the material has to be ground and sieved to obtained particles of an appropriate size range for subsequent use. This process is tedious and time-consuming, and the particles obtained are irregular in

size and shape, as well as part of the material is lost as fine dust. In addition, only 50% or less of the total amount of polymer is useful for analytical purposes, and some binding sites are partially destroyed during grinding which leads to a considerable loss of loading capacity of the imprinted polymer [12]. To overcome these problems, different approaches to synthesizing MIPs and IIPs have been proposed by several authors [31,32]. In accordance with Arshady [33], four different polymerization approaches (suspension, emulsion, dispersion and precipitation methods) can be carried out. In the first one, the initiator is soluble in the monomer, and these two are insoluble in the porogen. Therefore, there are two phases in which the monomer phase is suspended in the porogen by means of a stirrer and a suitable droplet stabilizer (suspension agent). Polymerization leads to the monomer "microdroplets" are converted directly to the corresponding polymer "microbeads" of approximately the same size. Emulsion polymerization uses a monomer insoluble in the porogen while the initiator is soluble in the polymerization medium and not in the monomer. This approach requires a surfactant in order to emulsify the two phases (monomer and porogen). Under these conditions, the monomer is present in the mixture partly in the form of droplets and partly in the form of micelles and a small percentage of it can also be molecularly dissolved in the porogen. Since the initiator is in the porogen, polymerization starts in the porogen and the formed oligoradicals are either surrounded by the dissolved monomer and the emulsifier molecules, or they are absorbed by the micelles. These emulsifier-stabilised structures gradually grow until the monomer is consumed. In either dispersion or precipitation polymerization methods the monomer and the initiator are both soluble in the porogen and the polymerization is initiated in homogenous solution and in the presence of a larger amount of porogen [34]. The difference between both methods is mainly attributed to the swollen capacity of the first primary particles. In the

dispersion method, the particles are swollen by the porogen and/or the monomer while in the precipitation method, the first particles do not swell in the solvent. For both methods, the polymer particles form a different phase (solid phase) depending on the solvency of the resulting macrocolecules (polymer particles) in the porogen. These techniques have emerged as attractive, simple and seemingly general methods for producing high-quality imprinted products, because crushing and sieving steps are avoided and higher yields of reaction are obtained. Application of dispersion polymerization to ionic imprinting can be found in the literature [22,23,25].

The aim of the current work has been the application of the precipitation polymerization approach to synthesizing different IIPs for nickel retention against major elements in seawater (mainly sodium and potassium). Different precipitation polymerization processes, involving only the template (Ni(II)) and the selected monomer (4-vinylpyridine, 4-VP, or 2-(diethylamino) ethyl methacrylate, DEM) or the vinylated monomer (4-VP or DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ), were carried out. For all cases, the synthesized polymer particles were packed into empty SPE cartridges, and they were evaluated / applied for nickel pre-concentration from seawater samples. Both ETAAS and ICP-OES have been used as selective detectors for nickel determination.

2. Experimental Section

2.1. Apparatus

A Perkin Elmer Model 1100B (Perkin Elmer, Norwalk, CT, USA) atomic absorption spectrometer equipped with an HGA-700 graphite furnace atomizer, deuterium background correction, an AS-70 auto-sampler and a nickel hollow cathode lamp (Cathodeon, Cambridge, U.K.) was used for the determination of nickel. An Optima

3300 DV inductively coupled plasma (ICP) atomic emission spectrometer (Perkin Elmer) equipped with an autosampler AS 91 (Perkin Elmer) and a Gem-Cone cross-flow nebulizer type (Perkin Elmer) was used for multi-elemental determinations. A temperature-controlled incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. A vacuum manifold station (Waters, Milford, MA, USA) connected to a vacuum pump (Millipore Co., Bedford, MA, USA) was used for SPE. IIPs were packed into 5 mL SPE cartridges (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA). ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Ultra-pure water of resistivity 18 MΩcm obtained from a Milli-Q purification device (Millipore Co.) was used to prepare all the solutions. High purity nitric acid and analytical grade $NiCl_2 \cdot 6H_2O$ were purchased from Panreac (Barcelona, Spain). Single standard solutions (1000 mg L^{-1}) of Ca, K, Mg, Na and Ni were from Merck (Darmstadt, Germany). High purity ammonia, ammonium chloride, and analytical grade 8-hydroxyquinoline were purchased from Merck. HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). 4-vinylpyridine (4-VP) and 2-(diethylamino) ethyl methacrylate (DEM) used as monomers were from Sigma-Aldrich (Steinheim, Switzerland). Divinylbenzene-80 (DVB) was from Sigma-Aldrich and was treated in order to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing around 0.5 g of neutral alumina (Sigma-Aldrich). 2,2′-azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at - 20°C after dissolving the reagent in methanol (Merck) at 50 – 60°C. After purification, this reagent was stored at

4°C. Estuarine seawater (SLEW–3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM–24 and TM-23.3) certified reference materials were purchased by the National Water Research Institute of Canada. All glass and plastic material were rigorously cleaned and kept into 10% (m/m) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before being used.

2.3. Seawater collection

Seawater samples were collected from the Ría de Muros-Noia estuary (north west Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were acidified at a pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then filtered through 0.45 μm polycarbonate membrane Nucleopore filters (Millipore) and stored at low temperature until used.

2.4. ETAAS measurements

Since Ni(II) was eluted from IIPs with a 2.0 M nitric acid solution, several experiments were carried out to determine the optimum temperatures and times for the charring and atomization steps using aqueous Ni(II) solutions (20 μg L^{-1}) in 2.0 M nitric acid. Since nickel is not a volatile element and as the sample solutions are free of salt, chemical modification was not considered. The optimized graphite furnace temperature program consisted of a drying step at 110°C (ramp rate and hold times of 10 and 20 s, respectively), followed by a charring stage at 1400°C (ramp rate and hold times of 30 and 10 s, respectively). Atomization was carried out at 2500°C (maximum power and 5 s as atomization/integration time). All experiments were carried out using pyrolytic coated graphite tubes with L'vov platforms and injection volumes of 20 μL. Negligible background signals were recorded under these optimized operating conditions. For all

nickel determinations, aqueous calibration in 2.0 M nitric acid was performed covering nickel concentrations until 50 μg L^{-1}.

2.5. ICP-OES measurements

Nickel and major elements such as sodium, potassium, calcium and magnesium were measured by ICP–OES (axial configuration) using a radiofrequency power of 1300 W and plasma, auxiliary and nebulizer argon flows of 15, 0.5 and 0.8 L min^{-1}. The detection wavelengths were 231.605 nm for Ni and 589.592, 766.490, 285.213 and 315.887 nm for Na, K, Mg and Ca, respectively. Determinations were performed by using aqueous standards in 2.0 M nitric acid. The calibration has covered Ni concentrations within the 0 - 4 mg L^{-1} range and up to 500 mg L^{-1} for Na, K, Ca and Mg.

2.6. Synthesis of nickel ionic imprinted polymer particles

Table 1 lists the monomers (vinylated reagents), non-vinylated ligand, cross-linker and free radical initiator as well as the amounts used in each synthesis. IIPs coded as IIP-1 and IIP-2 were synthesized in the absence of 8-HQ, DVB as a cross-linker and AIBN as an initiator. Solid $NiCl_2.6H_2O$ (~ 35 mg) was mixed with 4-VP (71.5 μL) or DEM (120 μL) into 15 mL glass test tubes. Then, 12.5 mL porogen (3:1 acetonitrile:toluene) was added and the mixture was stirred for 5 minutes, and then filtered. Finally, adequate volumes/amounts of DVB and AIBN (Table 1) were added, the glass tubes were purged with N_2 for 10 min at 0°C (tubes in an ice bath), were immediately sealed, and placed into a temperature-controllable incubator camera equipped with a low-profile roller. The roller allows the slow rotation of the tubes (33 r.p.m) about its long axis over the course of the polymerization. The temperature was ramped from room temperature to 60ºC over 2 h, and then maintained at 60ºC for a further 24 hours.

Table 1. Molar Ni(II)/Monomer/Ligand ratio and masses and volume of the different reagents involved into the polymerization process.

	Template ($NiCl_2 \cdot 6H_2O$)	Monomer (4-VP/DEM)	Ligand (8-HQ)	Cross linker (DVB)	Initiator (AIBN)	Molar ratio (Ni/Monomer/Ligand)	Mass of polymer[b]
IIP-1: Ni/4-VP	39.9 mg	71.5 µL		590 µL	46.6 mg	1/2/0	0.3121 g (62%)
IIP-2: Ni/DEM	35.5 mg	120 µL		530 µL	42.2 mg	1/2/0	0.3808 g (76%)
IIP-3: Ni/4-VP/8-HQ	38.3 mg	34.5 µL	46.7 mg	575 µL	45.46 mg	1/2/2	0.3935 g (79%)
IIP-4: Ni/DEM/8-HQ	36.4 mg	61.5µL	44.5 mg	545 µL	43.2 mg	1/2/2	0.4471 g (89%)
IIP-5: Ni/4-VP/8-HQ[a]	35.0 mg	31.5 µL	85.5 mg	525 µL	43.3 mg	1/2/4	0.3412 g (68%)
IIP-6: Ni/DEM/8-HQ[a]	33.4 mg	56.5 µL	81.7 mg	500 µL	41.3 mg	1/2/4	0.3000 g (60%)

(a) Double mass of 8-HQ

(b) Efficiency of the polymerization process (theoretical amount of 0.5 g) in brackets

IIPs coded as IIP-3 to IIP-6 were synthesized in the presence of a non-vinylated ligand (8-HQ). 8-HQ forms uncharged chelates with at least 60 elements [35,36] and offers as an advantage its lack of affinity for alkaline and alkaline earth metals. In this case, the template (~ 35 mg of $NiCl_2.6H_2O$) was mixed with 8-HQ (~ 45 mg for IIP-3 and IIP-4 or ~ 85 mg for IIP-5 and IIP-6) and the appropriate volume of monomer (4-VP or DEM) according to Table 1 and 12.5 mL of porogen. After stirring for 5 min the mixture was filtered, the cross-linker and initiator were added (Table 1) and the precipitation polymerization was carried out as described above. It must be noted that IIP-5 and IIP-6 were synthesized in the presence of a double amount of 8-HQ respect to IIP-3 and IIP-4. The ratio among the template (Ni(II)), monomer and ligand for each IIP is listed in Table 1. This table also gives the mass of polymer obtained and the efficiency of the polymerization process taking into account a theoretical amount of synthesized polymer of 0.5 g. It can be seen that efficiencies higher than 60% have been achieved for all cases.

The different IIPs were vacuum filtered, washed with acetonitrile, and then oven-dried overnight at 40ºC. Finally, the polymers (100 or 300 mg) were packed into 5 mL cartridges between Teflon frits.

Blank polymer particles (non-imprinted polymers, NIPs) were also prepared in the same way as IIPs, using the molar ratios listed in Table 1 but without the template. The NIPs were then subjected to the same washing pre-treatment as described in the following section.

2.7. Template removal procedure.

Once the imprinted polymer was prepared, the template (Ni(II) ions) must be removed from the polymer particles, leaving free cavities complementary in size, shape, and functionality ready for analyte recognition. Typically, template can be quantitatively

removed from the polymeric matrix by several hours of stirring the polymeric material with 1:1 hydrochloric acid [13] or other reagents such as acidic thiourea [25] or methanol/water and ethylenediaminotetracetic acid (EDTA) [23]. In the current work, the Ni(II) removal from the synthesized materials was carried out by extensively washing with 2.0 M nitric acid (5.0 mL aliquots) once the polymers were packed as SPE cartridges. Negligible nickel concentrations were found in the washing/filtrate solutions after passing 50 mL of 2.0 M nitric acid.

2.8. IIPs solid phase extraction

Nickel aqueous standard solutions were prepared in 100 mL of 0.1 M / 0.1 M NH_4Cl/NH_3 buffer solution at the convenient pH (optimum value of 8.5). Similarly, 100 mL of acidified seawater sample was treated with 1–2 mL of a 5.0 M ammonia solution, readjusting the pH to 8.5 ± 0.5. The use of ammonia or NH_4Cl/NH_3 buffer solutions is necessary in order to fix the pH and to prevent the transition metal hydroxides precipitation at high pHs. Then, the solutions were passed through cleaned and conditioned IIP cartridges at a fixed flow rate of 10 mL min^{-1} by using a vacuum manifold station. The cartridges were then rinsed with 2.5 mL of the NH_4Cl/NH_3 buffer solution at the same pH as that used for the loading solution, and then, the retained nickel ions were subsequently eluted with two 1.25 ml aliquots of 2.0 M nitric acid solution at a flow rate of 1.5 ml min^{-1}. A pre–concentration factor of 40 was achieved under these operating conditions. After elution, the IIPs were treated with 10 mL of Milli-Q water and then conditioned by passing 10 mL of the 0.1 M / 0.1 M NH_4Cl/NH_3 buffer solution at the working pH (8.5).

3. Results and Discussion

3.1. Preliminary evaluation of the synthesized polymers: Effect of pH.

A preliminary evaluation about imprinting properties of the synthesized polymers has been carried out by loading 600 ng of Ni(II) as 3.0 mL aliquots of 200 μg L^{-1} of Ni(II). These solutions were prepared in 0.1 M / 0.1 M NH_4Cl/NH_3 buffer solution at different pHs ranging from 4 to 9. After loading, the polymers were then rinsed with 3.0 mL of NH_4Cl/NH_3 buffer solution at a fixed pH (the same as the loading solution), and the retained nickel(II) ions were eluted with 3.0 mL of 2.0 M nitric acid. All experiments were carried out by triplicate and all solutions were analyzed by ICP-OES against an aqueous calibration in 2.0 M nitric acid. The effect of the pH on the nickel(II) retention was studied for all IIPs synthesized as well as all NIPs. Results as analytical recoveries are plotted in Figure 1A for those IIPs synthesized with 4-VP as a monomer and Figure 1B for IIPs based on the use of DEM as a monomer.

Figure 1A. Effect of the pH on the nickel analytical recovery for 4-VP based IIPs: IIP-1 (Ni/4-VP, 1/2), IIP-3 (Ni/4-VP/8-HQ, 1/2/2), IIP-5 (Ni/4-VP/8-HQ, 1/2/4), NIP-1 (4-VP), NIP-3 (4-VP/8-HQ, 2/2), NIP-5 (4-VP/8-HQ, 2/4).

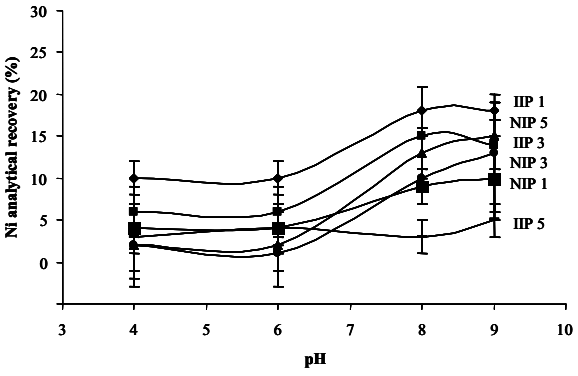
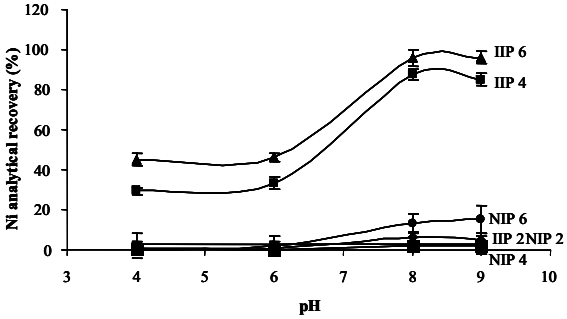
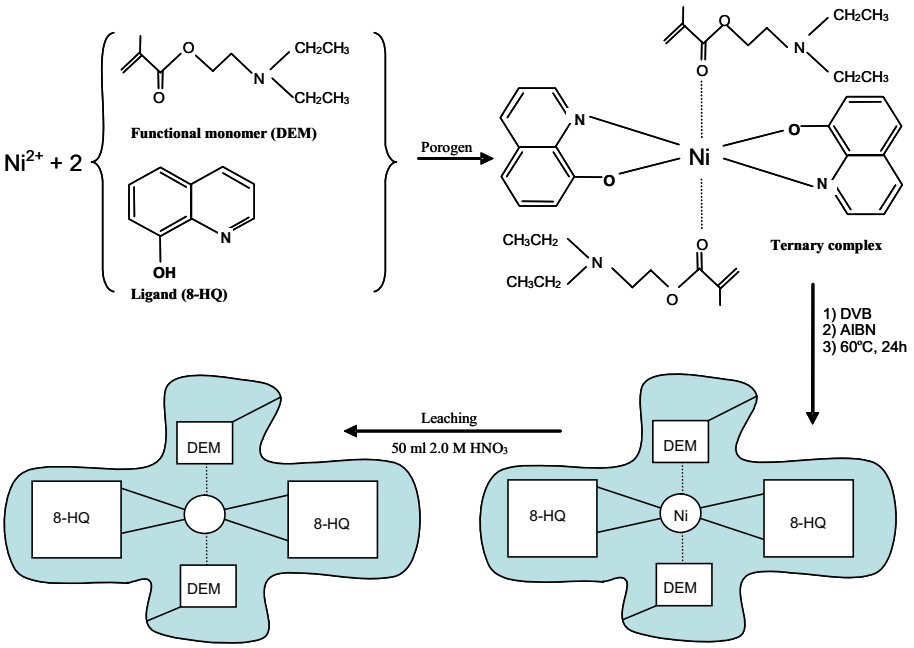


Figure 1B. Effect of the pH on the nickel analytical recovery for DEM based IIPs: IIP-2 (Ni/DEM, 1/2), IIP-4 (Ni/DEM/8-HQ, 1/2/2), IIP-6 (Ni/DEM/8-HQ, 1/2/4), NIP-2 (DEM), NIP-4 (DEM/8-HQ, 2/2), NIP-6 (DEM/8-HQ, 2/4).



It can be seen that IIP-4 and IIP-6 synthesized with DEM as a monomer and in the presence of 8-HQ gives good recoveries for nickel when working at high pHs (8-9). NIP-4 and NIP-6 have not shown affinity for nickel at any pH, which indicates that specific (imprinting) cavities were produced during the synthesis of IIP-4 and IIP-6. It must be noticed that IIP-2, synthesized with DEM but in the absence of 8-HQ has not shown imprinting properties for Ni. This result agrees with those reported by Metilda *et al*. for uranium pre-concentration, which showed that uranyl retention is only quantitative when using a IIP based on a ternary complex among uranyl ions, 5,7-dichloroquinoline-8-ol (DCQ) as a non-vinylated agent and 4-VP [37]. The synthesis of IIPs in the presence of DEM and 8-HQ leads to polymerization in the chemical bonding of the monomer, while the non-vinylated ligand is trapped inside the polymeric matrix. The proposed schematic representation of IIP-4 and IIP-6 synthesis is given in Figure 2.

Figure 2. Proposed schematic representation of the imprinting process for IIP-4 and IIP-6: dotted lines between Ni(II) ions and DEM show the possibility of a Ni-8-HQ-DEM ternary complex before polymerization.



Firstly, the template (Ni(II)) reacts with the ligand to form a nickel(II)-8-HQ binary complex and/or with the monomer and the ligand to form a nickel(II)-8-HQ-DEM ternary complex, which polymerize in the presence of DVB and AIBN through the vinyl groups of DEM [14]. In any case, the ligand (8-HQ) is trapped into the polymeric matrix and offers imprinting cavities for nickel(II). As recently reported, the non-vinylated agent is kept intact in the polymer matrix while leaching the template [13]. This fact have been observed for non-vinylated agents such as DCQ [37], dimethylglioxime and amino-, hydroxyl- or mercapto-quinolines [22] in the cross-linked polymers employing uranyl ions [37] and noble metals [22] as templates.

The trapped 8-HQ plays an important role in nickel(II) recognition because the optimum pH for loading (within 8 – 9) agrees with reported pHs for nickel-8-HQ complex formation in water [7]. The efficiency of 8-HQ to react with metal ions and form uncharged metal-8-HQ complexes is largely dependent on pH [35]. This is because 8-HQ is an ampholyte, forming oximium (8-hydroxyquinolinium) ion by protonation of N in acid solutions and oxinate ion in basic solutions. 8-HQ is only capable to interact with metal ions in basic solutions, because the hydroxyl group is not protonated [35].

Finally, IIP-1, IIP-3 and IIP-5, all synthesized in the presence of 4-VP as a monomer, have not shown imprinting properties for nickel (analytical recoveries lower than 20% and close similar to those achieved by using the corresponding NIPs).

3.2. Characterization studies

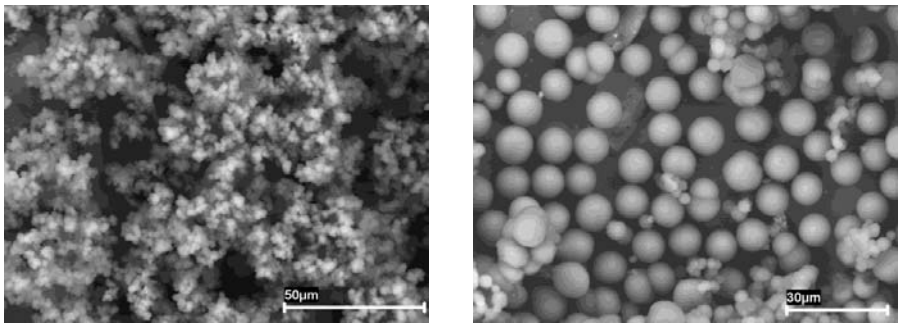
3.2.1. Scanning electron microscopy

In order to study the morphology and the size of the materials synthesized, scanning electron microscopy (SEM) pictures were taken from IIP-6 and NIP-6 (Figure 3). It can be seen that spherical monodisperse particles of around 10 μm of diameter were obtained for NIP-6, whereas IIP-6 consisted of agglomerates of different sizes formed by smaller particles. The different polymer morphology can only be attributed to the presence of the template, as previously reported in some works dealing with precipitation polymerization [38], and thus the existence of nickel(II)-8-HQ-DEM complexes leading to the formation of binding sites.

3.2.2. Energy dispersive X ray fluorescence studies

EDXRF patterns for IIP-6 material before and after leaching, as well as for the corresponding NIP-6 were obtained. It has been obtained that nickel was only present in the unleached polymer particles, and it was totally removed after leaching with 50 mL of 2.0 M nitric acid. It was also observed that the spectra of leached IIP-6 and NIP-6 were quite similar.

Figure 3. Scanning electron microscopy pictures for IIP-6 (A) and NIP-6 (B).



A B

3.2.3. Microanalysis studies

The elemental (H, C, N and O) composition of IIP-6 and NIP-6 were measured and values of 8.5% were found for hydrogen in both IIP-6 and NIP-6, while carbon was 85.6% and 88.2%, for IIP-6 and NIP-6, respectively. Nitrogen percentage was 3.5% and 3.1% for IIP-6 and NIP-6, respectively, and oxygen percentages were 3.3% and 2.8% for IIP-6 and NIP-6, respectively. The good agreement between calculated and experimentally found values of H, C, N and O can indicate that 8-HQ is indeed embedded in the polymeric matrix.

3.3. Optimization of nickel IIP-SPE from seawater

3.3.1. Effect of nitric acid volume / concentration for elution

Since Ni(II) ions are retained by binding to 8-HQ residues, elution of Ni(II) ions must imply the metal-8-HQ bonding destruction. This is easily reached by using acidic conditions. A first set of experiments were carried out in order to find the optimum nitric acid concentration for the eluting solution. 25 mL aliquots of Ni(II) aqueous standard solutions of 50 µg L^{-1} were prepared in 0.1 M / 0.1 M NH_4Cl/NH_3 buffer

solution at pH 8.5 and were passed through the cartridges loaded with 100 mg of IIP-6 under operating conditions shown in section 2.8. After rinsing, the retained analyte was eluted by passing volumes of 3.0 mL of nitric acid at concentrations between 2.0 and 5.0 M and these solutions were measured by ICP-OES against an aqueous calibration in 2.0 M nitric acid. The same analytical recoveries were obtained for all tested nitric acid concentrations, so an eluting solution of 2.0 M nitric acid was selected for further studies.

Similarly and in order to reach the highest pre-concentration factor, a volume of 2.5 mL of the eluting solution (2.0 M nitric acid) was tested. Results after ICP-OES measurement have shown that an efficient nickel elution is reached under both nitric acid volumes (2.5 and 3.0 mL). However, the elution process must be done in two steps. Quantitative nickel analytical recoveries were only obtained if nickel is eluted subsequently with two 1.5 mL aliquots (3.0 mL of the eluting solution) or 1.25 mL aliquots (2.5 mL of eluting solution). This fact could be attributed to the relatively high flow rate used around 1.5 $mLmin^{-1}$ which is the lowest flow rate allowed by the vacuum manifold station. A quantitative nickel elution could be reached using 3mL at once but at a lower flow rate. Since both eluting volumes have led to good analytical recoveries, an eluting volume of 2.5 mL was chosen in order to obtain the highest pre-concentration factor.

3.3.2. Effect of the load flow rate

Since a vacuum manifold station was used for IIP-SPE pre-concentration, the vacuum was fixed so that the sample solution as well as the rinsing and eluting solutions passed throughout the polymeric material at the lowest flow rate (around 1.5 mL min^{-1}). Other flow rates were tested and quantitative nickel recoveries were found for flow rates up to 10 mL min^{-1}. Therefore, this flow rate was chosen for further experiments.

3.3.3. Retention capacity

To determine the retention capacity (or sorption capacity) of the polymer (maximum amount of nickel ion retained from 1 gram of IIP), 100 mg of polymer were saturated with nickel ion under optimum conditions, by passing subsequently several 3.0 mL aliquots of 200 μg mL^{-1} Ni(II) solution, and measuring the nickel content in the eluates by ICP-OES. A retention capacity of the polymer was calculated to be 0.023 mmol g^{-1}. The theoretical retention capacity is 0.28 mmol g^{-1}; therefore, the calculated retention capacity is around 8% of the theoretical retention capacity. This result agrees with reported retention capacities for MIPs, around a 10% of the theoretical capacity [39].

3.3.4. Breakthrough volume: Effect of polymer mass

Two different cartridges were packed with different masses of IIP-6 polymer particles (100 mg and 300 mg). After adjusting the pH to 8.5 ± 0.5, different volumes (from 25 to 250 mL) of an aqueous solution containing 50 μg/L of nickel were passed through the cartridges at a flow rate of 10 mL min^{-1}. The retained nickel was then eluted with 2.5 mL of 2.0 M nitric acid and determined by ICP-OES. All experiments were performed by triplicate and the found nickel analytical recoveries are listed in Table 2. It can be seen that a polymer mass of 100 mg is not enough to reach quantitative nickel recoveries when using large sample volumes (larger than 25 mL). In this case, the breakthrough volume is 25 mL and when eluting with 2.5 mL of 2.0 M nitric acid, a maximum pre-concentration factor of 10 could only be achieved. However, larger volumes of loading solutions can be used when preparing IIP-SPE cartridges with 300 mg of polymeric material. In such cases, the breakthrough volume is not reached even after loading with 250 mL of sample solutions, and a pre-concentration factor of 100 can be obtained.

Table 2. Effect of polymer mass on breakthrough volume

Sample volume (mL)	Ni analytical Recovery (%)[a]	
	100 mg	300 mg
25	91 ± 2	101 ± 3
50	75 ± 5	98 ± 2
75	54 ± 6	97 ± 4
100	43 ± 4	96 ± 4
200	24 ± 6	94 ± 2
250	20 ± 4	92 ± 1

(a) n = 3

As a sensitive detector such as ETAAS is going to be further used to assess nickel in seawater samples, and to achieve a high pre-concentration factor in a reasonable period of time, a sample volume of 100 mL was chosen for a polymer mass of 300 mg, achieving a pre-concentration factor of 40, which is high enough to detect nickel in unpolluted seawater samples by ETAAS or ICP-OES.

3.3.5. Effect of major components from seawater

A set of experiments was carried out in order to observe interactions between the polymeric material and the major metals present in seawater (Na, K, Ca and Mg). A seawater sample was subjected eleven times to the proposed procedure and the concentrations of major ions were determined by ICP-OES. After pre-concentration, values around 30, 2, 10 and 5 mg L^{-1} were found for Na, K, Mg and Ca, respectively. These concentrations are very low taking into account the concentration of such elements in seawater (around 11490, 399, 1293 and 413 mg L^{-1}, for Na, K, Mg and Ca, respectively [40]). Therefore, it can be concluded that salt matrix is efficiently removed by using the IIP-6 and a selective pre-concentration of Ni is achieved.

3.4. Analytical performances for the nickel determination in seawater by IIP - SPE - ETAAS.

3.4.1. Calibration. Evaluation of matrix effect

Although IIP-SPE implies nickel separation from the seawater matrix, a comparison between calibration in 2.0 M nitric acid and standard addition was established in order to study a possible matrix effect. The standard addition graph was obtained after spiking four aliquots from a mixture of eluates, obtained after pre-concentration of a same seawater sample, with different nickel concentrations (between 0 and 30 $\mu g\ L^{-1}$). The aliquots were measured by ETAAS and 2.0 M nitric acid calibration and standard addition graphs were obtained. The mean and standard deviation for the slopes of three standard addition graphs (0.0046 ± 0.0006 A.u.Lμg^{-1}) and three external 2.0 M nitric acid calibrations (0.0049 ± 0.0004 A.u.Lμg^{-1}) were statistically compared by using the Cochran'C and Bartlett's tests at a 95.0% (comparison of variances), and the ANOVA test (comparison of means). It has been obtained that slopes for external 2.0 M nitric acid calibration and standard addition graphs are statistically comparable so the salt matrix was efficiently removed during the pre-concentration stage. This result agrees with no interaction between Na^+ and K^+ with the polymeric material (Section 3.3.6). Therefore, a simple calibration with nickel standard solution in 2.0 M nitric acid is adequate to perform seawater analysis for nickel. This fact offers a practical advantage so that a tedious and time consuming standard addition technique is not necessary. Most of the reported pre-concentration methods require the establishment of a standard addition graph of the overall SPE and analytical determination procedure [7].

3.4.2. Sensitivity of the method

Procedural blanks (i.e. 100mL of Milli-Q water subjected to the SPE procedure) were performed eleven times and the mean integrated absorbance (0.00906 A.u.) and

standard deviation (0.00894 A.u.) after ETAAS measurements were obtained. The limit of detection given by LOD = (3×SD)/m, where SD is the standard deviation of eleven procedural blanks, and m is the slope of the external 2.0 M nitric acid calibration graph, was calculated to be 137 ng L^{-1} for a pre-concentration factor of 40 and 55 ng L^{-1} for a pre-concentration factor of 100. Similarly, the limit of quantification, given by LOQ = (10×SD)/m (SD and m as above), was calculated to be 456 ng L^{-1} for a pre-concentration factor of 40 and 182 ng L^{-1} for a pre-concentration factor of 100. Such LOD and LOQ are low enough to determine trace nickel levels in unpolluted seawater samples, around 0.6 µg L^{-1} [40] and they are similar to those reported by other authors when using ETAAS as a detection technique and Amberlite XAD-4 [41] or Amberlite XAD-2 with Eriochorme blue black R as chelating agent [42], around 100 ng L^{-1}. LODs between 0.06 and 5.0 µg L^{-1} have been summarized by Praveen et al. [27] when using FAAS and different Amberlite sorbents / chelating agents. The use of C18 and ICP-OES detection showed a LOD value of 30 ng L^{-1} [7]. Finally, other sorbent materials based on IIPs have offered LODs of 5.0 µg L^{-1} for FAAS detection [27], 0.3 µg L^{-1} when using ETAAS [29], and 0.16 µg L^{-1} for ICP-OES measurements [30].

3.4.3. Repeatability and accuracy of the method

The repeatability of the overall procedure (IIP-SPE and ETAAS determination) was assessed by analyzing the same seawater sample eleven times. A relative standard deviation (R.S.D) of 6% was achieved for a mean nickel concentration of 1.62 µg L^{-1}, showing good repeatability of the overall procedure.

The accuracy of the method was verified by studying the analytical recovery and by analyzing different certified reference materials offering different salinities (SLEW-3, TM-23.3 and TM-24). Analytical recovery was assessed for three nickel concentration levels, after spiking three different aliquots from the same seawater sample with 1.0, 2.0 and 3.0 µg L^{-1} of Ni. Each nickel concentration was performed by triplicate; thus,

analytical recoveries are given as mean ± S.D. for three independent measurements (n = 3). These analytical recoveries are 104 ± 3 %, 98 ± 2 % and 99 ± 5 % for 1.0, 2.0 and 3.0 μg L^{-1} of Ni, respectively. It can be concluded that complete analytical recovery (within the 95 – 105 % range) was reached for all the nickel concentration levels.

SLEW-3 (estuarine water), TM-23.3 (lake water) and TM-24 (lake water) certified reference materials were analyzed in triplicate using a sample volume of 25 mL (pre-concentration factor of 10). Results, listed in Table 3, reveal good agreement between found concentrations and certified values for the three certified reference materials. This fact has been verified after applying the t-test for means comparison.

3.5. Application to real seawater samples

The optimized IIP-SPE-ETAAS method has been applied to five surface estuarine water samples from the Ría de Muros estuary (north-western Spain). All samples were sampled at the same sampling point in five different days. In addition, the method was applied to forty-five surface seawater samples from the A Coruña harbour (north-western Spain). In this case, samples were sampled at three different sampling points in fifteen different days. Each sample was subjected three times to the optimized procedure (pre-concentration factor of 40). Nickel concentrations in estuarine waters were between 1.21 ± 0.0442 μg L^{-1} and 1.70 ± 0.0350 μg L^{-1}, while nickel concentration between 0.480 ± 0.0103 μg L^{-1} and 6.52 ± 0.0305 μg L^{-1} were measured in seawaters from A Coruña harbour.

Table 3. Analysis of certified reference materials. Each material was analyzed by triplicate.

SLEW-3		TM-23.3		TM-24	
Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})
1.23 ± 0.07	1.30 ± 0.18	5.4 ± 0.6	5.2 ± 0.2	3.5 ± 3.0	3.4 ± 0.2

4. Conclusions

Nickel IIPs synthesized in the presence of DEM and 8-HQ have shown recognition capacities for nickel as well as quantitative pre-concentration of nickel(II) from seawater samples. Pre-concentration by solid phase extraction with IIP-6 particles results in a pre-concentration factor of 100 (250 mL of seawater sample and 2.5 mL of eluate), which offers a limit of detection of 50 ng L^{-1} by using ETAAS as a selective detector. The synthesized polymeric material has not offered affinity for major elements in seawater samples such as sodium and potassium, removing efficiently the salt matrix of seawater. This fact has been verified after statistical comparison of nickel aqueous standard calibrations in 2.0 M nitric acid and standard addition calibrations. Therefore, nickel measurements can be directly carried out using an aqueous calibration in 2.0 M nitric acid, being a fast method when coping with large number of samples. Finally, the IIP-SPE combined with ETAAS determination has offered accurate results for the analysis of nickel in lake water (low salinity) and estuarine water (high salinity).

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Inductively coupled plasma – optical emission spectrometry / mass spectrometry for the determination of Cu, Ni, Pb and Zn in seawater after ionic imprinted polymer based solid phase extraction

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Antonio Martin-Esteban

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RESUMEN

En el siguiente trabajo de investigación se ha evaluado la capacidad de un polímero de impronta iónica, originalmente preparado para la preconcentración de níquel en muestras de agua de mar, para reconocer otros elementos traza distintos del analito plantilla. El polímero ha sido sintetizado mediante la técnica de "polimerización por precipitación" utilizando un complejo de prepolimerización formado por la plantilla (Ni(II)), un monómero (DEM) y un agente complejante no vinilado (8-HQ). Dado que el agente complejante no posee grupos vinilo polimerizables, se encuentra atrapado en la matriz del polímero, pero no está unido químicamente a las cadenas poliméricas, por lo tanto, existe la posibilidad de que interaccione con otros compuestos iónicos, en lugar del Ni(II), originando la retención de dichos compuestos.

Los resultados obtenidos han demostrado que el polímero no solo reconoce el analito plantilla (Ni (II)) sino que, además, es capaz de reconocer el Cu (II), el Pb (II) y el Zn (II), con recuperaciones analíticas del 100% para todos estos elementos, mientras que el polímero blanco (Non Imprinted Polymer, NIP) no muestra afinidad por ninguno de ellos, probablemente porque los grupos funcionales de los monómeros y el agente complejante no se encuentran dispuestos en la posición geométrica adecuada.

Las variables que afectan al proceso de extracción en fase sólida de dichos elementos (pH de la muestra, velocidad de carga y de elución, concentración y volumen de la disolución de elución) han sido optimizados para la extracción simultanea de dichos metales, resultando como valores óptimos un pH de 8,5 ± 0,5, una velocidad de carga de 3 ml min^{-1}, una velocidad de elución inferior a 1,5 ml min^{-1} y un volumen de elución de 2,5 ml de ácido nítrico 2,0 M.

La exactitud del método para los diferentes metales adsorbidos fue evaluada mediante la realización de un estudio de recuperación analítica y mediante el análisis de diferentes materiales de referencia certificados (SLEW-3, TM-23.3 y TM-24). También se estudió la precisión del método mediante un estudio de reproducibilidad (n=11), siendo empleados el ICP-OES y ICP-MS como técnicas multielementales de detección.

Después de pasar 250 ml de una muestra de agua de mar se obtiene un factor de preconcentración de 100, originando unos los límites de detección (LOD) de 0,14, 0,15, 0,18 y 0,03 $\mu g\ l^{-1}$ (ICP-OES) y 0,0022, 0,0065, 0,0040 y 0,009 $\mu g\ l^{-1}$ (ICP-MS) para el Ni, el Cu, el Pb y el Zn, respectivamente. Además, el polímero no interacciona con los metales alcalinos (Na^{+}, K^{+}) ni alcalinotérreos (Mg^{2+}, Ca^{2+}); por lo tanto, la matriz salina del agua de mar puede ser eficientemente eliminada, realizándose la determinación de los analitos mediante el establecimiento de una sencilla recta de calibrado. Además, el polímero sintetizado puede ser reutilizado, al menos, 25 veces sin perder sus propiedades adsorbentes.

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Inductively coupled plasma – optical emission spectrometry / mass spectrometry for the determination of Cu, Ni, Pb and Zn in seawater after ionic imprinted polymer based solid phase extraction

Jacobo Otero-Romaní[1], Antonio Moreda-Piñeiro[1], Pilar Bermejo-Barrera[1], Antonio Martin-Esteban[2]

(1) Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain. (2) Department o Environment. National Institute for Agriculture and Food Research (INIA). Carretera de A Coruña, Km 7,5. 28040 – Madrid. Spain.

Abstract

The capabilities of a synthesized ionic imprinted polymer (IIP), originally prepared for Ni recognition/pre-concentration from seawater, has been evaluated for other trace elements pre-concentration. The polymer has been synthesized by the precipitation polymerization technique using a ternary pre-polymerization complex formed by the template (Ni), the monomer (2-(diethylamino) ethyl methacrylate, DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ). Since the complexing agent (8-HQ) is trapped into the polymeric matrix, but is not linked to the polymer chains, specific interactions between the functional groups (present in the monomer and the complexing agent) and other trace elements rather than Ni may occur.

Results have shown that the IIP offers imprinting properties for the template (Ni(II)) and also for Cu(II), Pb(II), Zn(II), As(V) and Cd(II), with analytical recoveries close to 100% for all elements except for As(V) and Cd(II) (around 70%), whereas the non-imprinted polymer (NIP) did not show affinity for any trace element. In addition, the

polymer does not interact with alkaline or alkaline-earth metals, so Na, K, Mg and Ca from the seawater salt matrix could be effectively removed. Variables affecting the IIP-solid phase extraction (SPE) process (pH, load flow rate and concentration and volume of the eluting solution) were completely studied. Inductively coupled plasma – optical emission spectrometry (ICP-OES) and inductively coupled plasma – mass spectrometry (ICP-MS) have been used as multi-element detectors.

Acidified seawater samples must only be treated to fix an alkaline pH (8.5±0.5) and passed through IIP-SPE cartridges. After seawater sample loading (250 mL), analytes were eluted with 2.5mL of 2.0M nitric acid, offering a pre-concentration factor of 100. Therefore, the limits of detection (LODs) of the method were 0.14, 0.15, 0.18 and 0.03 μg L^{-1}, for Ni, Cu, Pb and Zn, respectively, when using ICP-OES detection and 0.0022, 0.0065, 0.0040 and 0.009 μg L^{-1}, for Ni, Cu, Pb and Zn, respectively, for ICP-MS detection. Accuracy of the method was assessed by analyzing SLEW-3 (estuarine water) and TM-23.3 and TM-24 (lake water) certified reference materials.

Keywords

Ionic imprinted polymer, copper, nickel, lead, zinc, seawater, solid phase extraction, inductively coupled plasma - optical emission spectrometry, inductively coupled plasma - mass spectrometry

1. Introduction

The protection of marine and continental waters against contamination has increased in recent years to allow adequate conditions for aquatic life and a responsible use of such natural resources. The European Union has therefore established several Directives on the quality of continental and marine waters and for marine ecosystems. These

European Directives limit the levels of some trace elements such as Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in seawater used for mollusk production [1]. Therefore, the development of fast and reliable analytical methods to assess trace elements in water is a current topic. This fact is especially important for seawater analysis because of the high salt content which is a source of spectral and matrix interferences [2,3] and because most of the target metals are present at very low levels [4].

Solid phase extraction (SPE) is, by far, one of the most used sample pre-treatments for complex matrices such as seawater, mainly because trace analyte pre-concentration and sample matrix (salts) removal are achieved at time [5]. Bonded silica sorbents are used in typical SPE procedures for organic compound pre-concentration and also for trace elements [6,7,8]. However, it has been reported that interfering compounds are often co-extracted with the target analytes and a matrix effect can be observed using these conventional sorbents [6]. Therefore, more selective systems for separation of trace elements are required and the development or synthesis of new sorbents has increased in recent years [9]. High selectivity can be expected from sorbents such as inmunosorbents (ISs) and molecularly imprinted polymers (MIPs) [10]. However, the main advantage of MIPs over ISs is the fast and less expensive synthesis and the high degree of molecular recognition. Synthesized sorbents in the presence of ions, instead of molecules, as templates generate ionic imprinted polymers (IIPs), which offer all the benefits derived from MIPs and a high capacity for recognizing ions.

Ionic imprinting polymerization is based on the preparation of a highly cross linked polymer around an ion (the template) in the presence of a suitable monomer (vinylated reagent) or in the presence of a monomer and an auxiliary / complexing non vinylated reagent [11,12]. As recently reviewed by Rao et al. [12], there are four different approaches for the synthesis of metal ion-imprinted polymers, viz. (i) linear chain

polymers carrying metal-binding groups being cross-linked with a bifunctional reagent; (ii) chemical immobilization by preparation of binary complexes of metal ions with ligands having vinyl groups, isolation and then polymerization with matrix-forming monomers; (iii) surface imprinting conducted on an aqueous-organic interface; (iv) trapping of non-vinylated chelating ligand via imprinting of binary/ternary mixed ligand complexes of metal ions with non-vinylated chelating agent and vinyl ligand. The first three approaches use vinylated reagents, which form complexes with the template (metal ion) and can polymerize through the vinyl groups. Therefore, the complexing ligand is chemically immobilized in the polymeric matrix. The main drawback of these approaches is the fact that complexing ligands showing vinyl groups are scarce and are not available, so the synthesis of these complexing monomers must be performed in the laboratory. Some examples of these complexing monomers are those based on amino acids such as mathacryloylhistidine (MAH) [9,13], and N-methacryloly-(l)-cysteinemethylester (MAC) [14,15,16], or N-methacryloyl-L-glutamic acid (MAGA) [17], which have been widely used for recognizing Al [17], Cd [14,15], Cu [13], Fe [16] and Ni [9]. Other ligands such as (2Z)-N,N'-bis(2-aminoethylic)but-2-enediamide for Cd [18] and o-phenylenediamine for Se [19] have also been reported as bifunctional monomers.

The use of special synthesized vinylated ligands can be avoided when using the trapping technique. In this case, a ternary monomer-non vynilated ligand-metal complex is formed; therefore, the number of useful ligands is large because most of the metal complexing agents are not vinylated reagents. After polymerization, the non vinylated ligand is not chemically bonded to the polymer chains, but instead is trapped inside the polymeric matrix. By using the trapping approach, different non-vinylated reagents such dimethylglyoxime for recognizing palladium [20], diazoaminobenzene [21] for

recognizing mercury, cetyltrimethyl ammonium bromide [22] for recognizing Cd and Zn, and derivates of quinoline, mainly 5,7-dichloroquinoline-8-ol for lantanides [23,24,25,26], Pd [27], U [28,29,30] and Co and Ni [31] have been successfully applied. The aim of the current work has been the study of the capabilities of a synthesized ionic imprinted polymer, originally prepared for Ni recognition/pre-concentration from seawater, for other trace elements pre-concentration. The polymer has been synthesized by the precipitation polymerization technique using a ternary pre-polymerization complex formed by the template (Ni), the monomer (2-(diethylamino) ethyl methacrylate, DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ). Inductively coupled plasma – optical emission spectrometry (ICP-OES) and inductively coupled plasma – mass spectrometry (ICP-MS) have been used as multi-element detectors.

2. Experimental Section

2.1. Instrumentation

Optima 3300 DV inductively coupled plasma atomic emission spectrometer (Perkin Elmer, Norwalk, CT, USA) equipped with an autosampler AS 91 (Perkin Elmer) and a Gem-Cone cross-flow nebulizer type (Perkin Elmer) was used for multi-elemental determinations. 820-MS inductively coupled plasma quadrupole mass spectrometer (Varian, Mulgrave, Australia) equipped with an SPS3 autosampler (Varian) and a MicroMist nebulizer type (Varian). Temperature-controllable incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. Vacuum manifold station (Waters, Milford, MA, USA) connected to a vacuum pump (Millipore Co., Bedford, MA, USA) was used for SPE. IIPs were packed into 5ml syringes (Brand, Wertheim, Germany)

between replacement Teflon frits (Supelco, Bellefonte, PA, USA). ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Ultra-pure water of resistivity 18MΩcm obtained from a Milli-Q purification device (Millipore Co.) was used to prepare all the solutions. 70% nitric acid and analytical grade $NiCl_2 \cdot 6H_2O$ were purchased from Panreac (Barcelona, Spain). Single standard solutions (1000 mg L^{-1}) of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn were from Merck (Darmstadt, Germany). Ammonia 25%(v/v), ammonium chloride, and 8-hydroxyquinoline were purchased from Merck. HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). 2-(diethylamino) ethyl methacrylate (DEM) used as monomer was from Sigma-Aldrich (Steinheim, Switzerland). Divinylbenzene-80 (DVB) from Sigma-Aldrich was treated in order to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing around 0.5g of neutral alumina (Sigma-Aldrich). 2,2′-azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at -20°C after dissolving the reagent in methanol (Merck) at 50 – 60°C. After purification, this reagent was stored at 4°C. Estuarine seawater (SLEW–3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM–24 and TM-23.3) certified reference materials were purchased from the National Water Research Institute of Canada. All glass and plastic material was rigorously cleaned and kept into 10% (v/v) nitric acid for at least 48h. The material was then rinsed three times with ultra-pure water before being used.

Table 1. Operating ICP–OES conditions

General	Radiofrequency power / W	1300
	Sample uptake rate / mL min^{-1}	1.5
	Stabilization delay / s	45
	Number of replicates	4
	Integration time / s	5
	Nebulizer type	Cross flow
	Axial view	
Gas flows / L min^{-1}		
	Plasma	15.0
	Auxiliary	0.5
	Nebulizer	0.8
Detection wavelengths / nm		
	Cu	327.393
	Ni	231.604
	Pb	224.688
	Zn	213.857

2.3. Seawater collection

Seawater samples (1L) were collected from the Ría de Muros-Noia estuary (north-western Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were filtered through 0.45μm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then and stored at low temperature until used.

2.4. ICP-OES/MS measurements

Multi-element determinations (As, Cd, Cu, Ni, Pb and Zn) were performed by ICP–OES (axial configuration) using the operating conditions and emission wavelength lines given in Table 1. Determinations were performed by using aqueous standards in 2.0M nitric acid covering metal concentrations within the 0 – 0.25 mg L^{-1} range for As, Cd, Cu, Ni and Pb, and between 0 and 1.0 mg L^{-1} for Zn.

Similarly, As, Cd, Cu, Ni, Pb and Zn were measured by ICP–MS under operating conditions listed in Table 2. The use of H_2 in the collision cell at a flow rate of 80 mL min^{-1} gave the best sensitivity as well as minimised possible polyatomic interferences for the target elements [32].

Table 2. Operating ICP–MS conditions

General		
	Radiofrequency power / W	1400
	Sample uptake rate / r.p.m.	3.0
	Stabilization delay / s	35
	Number of replicates	3
	Integration time / s	5
	Nebulizer type	MicroMist
Gas flows / L min^{-1}	Plasma	17.0
	Auxiliary	1.65
	Sheath	0.27
	Nebulizer	0.99
Torch alignment / mm		
	Sampling death	7.0
Ion optics / V		
	First extraction lens	-32
	Second extraction lens	-164
	Third extraction lens	-231
	Corner lens	-206
	Mirror lens right	25
	Mirror lens left	24
	Mirror lens bottom	27
	Entrance lens	3
	Fringe Bias	-4.9
	Entrance plate	-3.4
	Pole bias	0
CRI / mL min^{-1}		
	Skimmer gas source	H_2
	Sampler gas source	OFF
	Skimmer flow	80
	Sampler flow	0
Mass-to-ratio		
	Cu	63
	Ni	60
	Pb	208
	Zn	66

This flow was optimized by monitoring the counts for a 2.0M nitric acid eluate containing the target elements. Finally, determinations were performed by using aqueous standards in 2.0M nitric acid with metal concentrations from 0 to 0.25 mg L^{-1} (As, Cd, Cu, Ni and Pb) and Zn concentrations from 0 to 1.0 mg L^{-1}.

2.5. Synthesis of nickel ionic imprinted polymer particles

0,0334g of $NiCl_2 \cdot 6H_2O$, 56.5μL of DEM and 0,0817g of 8-HQ were mixed in 12.5mL of porogen (3:1 acetonitrile:toluene). This solution was stirred for 5min and then filtrated. Finally, 500μL of DVB (cross-linker) and 41.3mg of AIBN (initiator) were added, the glass tubes purged with N_2 and immediately sealed just before thermal

induction of the precipitation polymerization. The temperature was ramped from room temperature to 60ºC over 2h, and then maintained at 60ºC for a further 24h. Always, a low stirring rate of 33r.p.m. was used. The polymer was vacuum filtered, washed with acetonitrile and then oven-dried overnight at 40ºC. Finally, the polymer (around 300mg) was packed into 5mL cartridges between Teflon frits. It should be mentioned that the polymer was synthesized in presence of a double amount of 8-HQ with respect to DEM. This gives a Ni/DEM/8-HQ molar ratio of 1/2/4. The mass of polymer obtained after polymerization was 0.300g and the efficiency of the polymerization process, taking into account a theoretical amount of synthesized polymer of 0.5g, was 60%. The template (Ni(II) ions) were removed from the polymer particles by extensive washing with 2.0M nitric acid (5.0mL aliquots). Negligible nickel (template) concentrations by ICP-MS in the washing/filtrate solutions were found after passing 50mL of the washing solution. Blank polymer particles (non imprinted polymer, NIP) were also prepared in the same way than IIP, but without the template. The NIP was then subjected to the same washing pre-treatment as described above.

The synthesized materials (IIP and NIP) were characterized by scanning electron microscopy (SEM), energy dispersive X ray fluorescence (EDXRF) and microanalysis (elemental H, C, N and O composition). This information is given elsewhere [35].

2.6. IIP solid phase extraction

Multi-element aqueous standard solutions were prepared in 100 or 250mL of 0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 8.5±0.5. Similarly, 100 or 250mL of acidified seawater samples were treated with volumes of a 5.0M ammonia solution (with the 100 - 500 μL range), to readjust the pH to 8.5±0.5. Then, the solutions were passed through cleaned and conditioned IIP cartridges at a flow rate of 3.0 mL min^{-1} by using a vacuum manifold station. The cartridges were then rinsed with 2.5 mL of the

0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 8.5±0.5, and then, the retained ions were subsequently eluted with two 1.25mL aliquots of 2.0M nitric acid solution at a flow rate of 1.5 mL min^{-1}. A pre–concentration factor of 40 or 100 was achieved under these operating conditions. After elution, the IIPs were treated with 10mL of Milli-Q water and then conditioned by passing 10mL of the 0.1M/0.1M NH_4Cl/NH_3 buffer solution at the working pH (8.5).

Blanks of the procedure were also obtained by applying the SPE procedure above described. In this case, around 1L of Milli-Q water was acidified by adding 1.0mL of concentrated nitric acid (pH lower than 2), and then 100mL aliquot samples were treated with the buffer solution to fix the pH 8.5±0.5, and were subjected to the SPE procedure.

3. Results and discussion

3.1 Optimization of trace elements IIP-SPE from seawater

3.1.1. Effect of pH

A set of experiments was carried out by applying the pre-concentration procedure (section 2.6) to 100mL aliquots of an aqueous solution containing the template, Ni(II), and other metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Pb, Sn, Ti, V and Zn) at 50 µg L^{-1} each one. The same experiments were carried out by using the NIP. The different pHs tested (from 4.0 to 9.0) were fixed by using 0.1M/0.1M NH_4Cl/NH_3 buffer solutions.

Three replicate SPE were performed at each pH and after ICP-OES detection, the polymeric material offered specific interactions for Ni and also for Cu(II), Pb(II), Zn(II), As(III) and Cd(II) since the analytical recoveries for the non-imprinted polymer are quite lower than when using the IIP for most of the tested pHs. Other trace elements were not specifically retained at any pH.

Figure 1. Effect of the pH on the nickel (a), copper (b), lead (c), zinc (d), arsenic (e) and cadmium (f) analytical recovery after solid phase extraction with an ionic imprinted polymer (IIP) and a blank or non imprinted polymer (NIP).

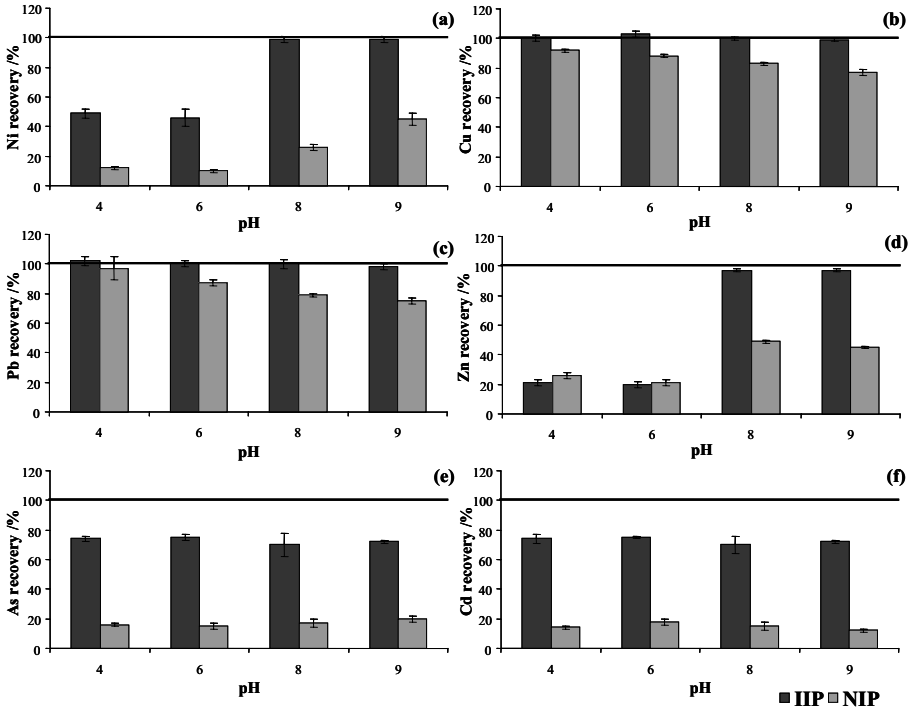

Figure 1 shows the effect of the pH on the analytical recovery of Ni, Cu, Pb, Zn, As and Cd after IIP/NIP-SPE. It can be observed that Ni and Zn are only quantitative retained in the IIP at pHs in-between 8.0 and 9.0, whereas Cu and Pb are quantitative retained at all tested pHs. As and Cd show analytical recoveries near 70% at all pHs tested.

The fact that other ions in addition to the template (Ni(II)) interact with the specific cavities formed for nickel must be explained keeping in mind the presence of the non-vinylated agent (8-HQ). After polymerization, this agent is trapped into the polymeric matrix and the specific cavities formed for nickel during the polymerization are

influenced by the presence of 8-HQ which can interact with other ions, although the shape and / or size of such ions was different to the template. The pre-concentrating capacities of the synthesized polymer for Ni(II), Cu(II), Pb(II) and Zn(II) is in agreement with other pre-concentration studies based on resins-immobilized 8-HQ, such as those reported by Howard et al. for silica-immobilized 8-HQ [33], and by Askun et al. for 8-HQ anchored poly(styrene-divinylbenzene) microbeads [34]. For both supports, the same divalent trace elements (Cu, Ni and Zn [33], and Pb, Cd, Ni and Co [34]) were selectively pre-concentrated. This selectivity must be related to a metal complexation with the immobilized 8-HQ because many elements are complexed with 8-HQ in solution [6]. Therefore, the "flexibility" of the imprinting cavities, attributed to 8-HQ, gives the possibility of using this IIP for the selective pre-concentration of a certain number of elements: Cu, Ni, Pb and Zn. From the previous studies (Figure 1), a compromise pH of 8.5±0.5 was chosen to achieve the highest analytical recovery for this group of elements.

3.1.2. Effect of the load flow rate

Different experiments were performed in order to find the optimum load flow rate for the sample solutions. Different aliquots from the same seawater sample were passed subsequently at 1.5, 3.0, 6.0 and 12 mL min^{-1}. The flow rates were fixed by using a vacuum manifold station. Results have shown the same As, Cd, Ni and Zn concentrations for all flow rates tested; however, Cu and Pb concentrations decrease for flow rates larger than 3 mL min^{-1}. In order to achieve the highest analytical recovery in the lowest period of time, a 3 mL min^{-1} load flow rate was chosen, and used for further experiments.

3.1.3. Effect of nitric acid volume / concentration for elution

Different experiments were performed in order to find the optimum nitric acid concentration / volume for the eluting solution. After sample loading and rinsing (section 2.6), the retained analytes were eluted by passing volumes of 2.5mL of nitric acid at concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0M at a flow rate of 1.5 mL min^{-1}. After measuring by ICP-OES against an aqueous calibration in 1.0, 2.0, 3.0, 4.0 or 5.0M nitric acid, quantitative analytical recoveries were obtained for all cases; thus, an eluting solution of 2.0M nitric acid was selected for further studies.

Regarding the volume of the eluting solution, volumes of 2.5 and 3.0mL of a 2.0M nitric acid solution were tested. Experiments by triplicate at a fixed flow rate of 1.5 mL min^{-1} have shown that an efficient metal elution is reached under both nitric acid volumes, although the elution process must be done in two steps. Therefore, analytes were eluted subsequently with two 1.5mL aliquots (3.0mL of the eluting solution) or 1.25mL aliquots (2.5mL of the eluting solution). In order to achieve the highest pre-concentration factor, an eluting volume of 2.5mL was chosen (pre-concentration factor of 100 for a seawater sample volume of 250mL).

3.2. Effect of major components from seawater

Different experiments were carried out to observe interactions between the polymeric material and the major metals present in seawater (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}). To check possible interaction of the major cations, a seawater sample was subjected eleven times to the proposed procedure and the concentrations of major ions were determined by ICP-OES. After pre-concentration (factor pre-concentration of 100), values around 30, 2, 10 and 5 mg L^{-1} were found for Na, K, Mg and Ca, respectively. These concentrations are very low taking into account the concentration of such elements in seawater (around 11490, 399, 1293 and 413 mg L^{-1}, for Na, K, Mg and Ca, respectively [4]).

To check possible retention of chloride and sulphate, a seawater sample was subjected eleven times to the proposed procedure (pre-concentration factor of 100) and the nitric acid eluates were measured by ICP-MS (m/z ratios of 32 and 35 for sulphur and chlorine, respectively). Negligible signals for both analyte were obtained, which means they are not retained in the polymeric matrices or that potential polyatomic interferences are avoided by using collision cell. Therefore, it can be concluded that salt matrix is efficiently removed while a selective pre-concentration of Ni, Cu, Pb and Zn is achieved.

3.3. Cross reactivity

Different experiments were conducted in order to evaluate the cross-reactivity among nickel and other selected trace elements (Al, As, Cd, Cr, Cu, Mn, Pb, Sn, Ti, V, and Zn). 100 mL aliquots of an aqueous standard solution at pH to 8.5 ± 0.5 containing 50 $\mu g\ l^{-1}$ of nickel and other trace metals were subjected three times to the optimized SPE procedures by using cartridges prepared with IIP and NIP materials. After elution and ICP-OES measurements, parameters such as extraction efficiency, distribution ratio (D) and selectivity coefficient ($S_{Ni/M}$), defined as shown in Table 3, were calculated. As it was expected, results (Table 3) show that the polymeric material (IIP) does not discriminate between Ni, Cu, Pb and Zn, as they remain linked to the polymer (percent extraction higher than 99 %) and only detach after acidic elution. It can be concluded that the polymer offers imprinting properties for Ni (the template) and also for Cu, Pb and Zn whereas the NIP material did not show affinity for Ni, Cu, Pb or Zn (percent extraction within 10 to 27 %), indicating that those specific cavities formed for nickel during the IIP polymerization are responsible of Ni, Cu, Pb and Zn retention. The IIP material is specific for Ni, Cu, Pb and Zn, and other transition metals (Cr, Sn, Ti, V, As, Cd, Mn and Al) are not retained.

Table 3. Extraction (%), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$) of for IIP and NIP

	Ionic imprinted polymer (IIP)		
Element	Extraction (%)[a]	Distribution ratio (D)[b]	Selectivity coefficient ($S_{Ni/M}$)[c]
Ni	99	99	-
Cu	99	99	1.0
Pb	99	99	1.0
Zn	99	99	1.0
Cr	80	4.0	25
Sn	78	3.5	28
Ti	80	4.0	25
V	21	0.3	372
As	72	2.6	38
Cd	71	2.4	40
Mn	16	0.2	520
Al	24	0.3	314
	Non-ionic imprinted polymer (NIP)		
Ni	10	0.1	-
Cu	23	0.3	0.4
Pb	27	0.4	0.3
Zn	19	0.2	05
Cr	75	3.0	0.04
Sn	73	3.0	0.04
Ti	79	3.8	0.03
V	21	0.3	0.42
As	14	0.2	0.68
Cd	14	0.2	0.68
Mn	12	0.1	0.81
Al	21	0.3	0.42

(a) $\% = (A_2 / A_T) \times 100$

(b) $D = (A_2 / A_1)$

(c) $S_{Ni/M} = D_{Ni} / D_M$

A_1 = Amount of metal ion in aqueous solution at equilibrium

A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium.

A_T = Total amount of metal ion used in extraction.

D_{Ni} = Distribution ratio for Ni

D_M = Distribution ratio for M (M = Cu, Pb, Zn, Cr, Sn, Ti, V, As, Cd, Mn and Al)

3.4. Analytical performances for the IIP-SPE- ICP-OES/MS procedures

3.4.1. Calibration. Evaluation of matrix effect

Three different calibration graphs were performed: (1) external aqueous calibrations in 2.0M nitric acid; (2) standard addition calibrations, obtained after spiking a pool of nitric acid eluates from different 100mL aliquots from the same seawater sample with the target elements; and (3) standard addition calibrations prepared following the IIP-SPE pre-concentration procedure (100mL seawater sample spiked with 1.0, 2.0 and 4.0µg L^{-1} for As, Cd, Cu, Ni and Pb and 5, 10 and 20 µg L^{-1} for Zn, and subjected to the IIP-SPE procedure (section 2.6)). External aqueous calibrations and standard addition calibrations were tested covering metal concentrations between 0 and 0.25 mg L^{-1} (As, Cd, Cu, Ni and Pb) and between 0 and 1.0 mg L^{-1} (Zn).

For all cases, three different external aqueous calibrations, standard addition calibrations and "procedure" addition calibrations were performed in different days. The mean and the standard deviation of the slopes (ICP-OES and ICP-MS) are given in Table 4. After statistical comparison of the means by the multiple range test (95% confidence range), it has been obtained that slopes for external aqueous calibration in 2.0M nitric acid and standard addition calibrations are statistically comparable for all elements when using either ICP-OES and ICP-MS.

Table 4. Means and standard deviations (mean ± S.D.) for aqueous calibration in 2.0M nitric acid, standard addition and standard addition calibrations prepared following the IIP-SPE pre-concentration procedure (N = 3)

	ICP-OES[a]		
	Aqueous calibration	Standard addition	Standard addition calibration following the IIP-SPE
Ni	6.8 ± 0.1	6.6 ± 0.1	6.6 ± 0.2
Cu	63 ± 2	62 ± 2	61 ± 1
Pb	3.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.2
Zn	10 ± 0.2	10 ± 0.1	9.9 ± 0.1
As	6.5 ± 0.3	6.1 ± 0.2	3.7 ± 0.2
Cd	6.1 ± 0.1	6.0 ± 0.1	3.7 ± 0.2
	ICP-MS[b]		
	Aqueous calibration	Standard addition	Standard addition calibration following the IIP-SPE
Ni	545 ± 35	530 ± 27	496 ± 27
Cu	1394 ± 85	1385 ± 71	1291 ± 111
Pb	74754 ± 587	71825 ± 1115	70078 ± 4192
Zn	9137 ± 255	9195 ± 366	9049 ± 709
As	364 ± 4	362 ± 14	63 ± 1
Cd	3544 ± 287	3467 ± 167	2225 ± 192

(a) L mg^{-1}

(b) L μg^{-1}

Therefore, it can be concluded that salt matrix is efficiently removed during the pre-concentration stage. This result agrees with the fact that there is no interaction between Na and K with the polymeric material [35]. In addition, slopes of the external aqueous calibration in 2.0M nitric acid and the "procedure" addition calibrations are also statistically similar (95% confidence range), for those elements which were

quantitatively retained by the polymer (Ni, Cu, Pb and Zn). The slopes for other elements (As and Cd), which show specific interactions but non-quantitative retention, were not statistically comparable. The same results have been obtained when comparing the standard addition calibrations and the standard addition calibration prepared following the IIP-SPE pre-concentration procedure. Therefore, an external aqueous calibration in 2.0M nitric acid can be used to determine Ni, Cu, Pb and Zn in seawater after IIP-SPE, but a standard addition calibration prepared following the IIP-SPE pre-concentration procedure is needed for the quantitative determination of As and Cd in the nitric acid eluates after IIP-SPE.

The feasibility of external aqueous calibration offers a practical advantage so that tedious and time consuming standard addition techniques are not necessary. It should be mentioned that most of the reported pre-concentration methods require the establishment of a standard addition graph of the overall SPE and analytical determination procedure [8], so the synthesized polymer is advantageous for Ni, Cu, Pb and Zn pre-concentration/determination in seawater.

3.4.2. Sensitivity of the method

The limits of detection and quantification were calculated according to LOD = (3*SD)/m and LOD = (10*SD)/m, where SD is the standard deviation of eleven measurements of a procedural blank (acidified Milli-Q water treated as a sample) and m is the slope of the external 2.0M nitric acid calibration graph. Values after a pre-concentration factor of 100 are listed in Table 5. Such LODs and LOQs are low enough to determine Ni, Cu, Pb and Zn levels in unpolluted seawater samples. These values are similar to those reported by other authors when using ICP-MS (0.01 μg L^{-1} for Ni using Amberlite XAD-4 immobilized butane-2,3-dione bis(N-pyridimoacetylhydrazone) [36], and from 0.0001 μg L^{-1} (Pb) to 0.001 μg L^{-1} (Ni) for Chelex 100 down-sized packed minicolumns [37]), ICP-OES (values from 0.02 to 1.13 μg L–1 for the use of C18 [6]) and ETAAS (0.10 μg L^{-1} for Ni using Amberlite XAD-4 [38] or Amberlite XAD-2 with Eriochorme blue black R as chelating agent [39]).

Table 5. LOD and LOQ of the methods.

	ICP-OES	
	LOD / μg L^{-1}	LOQ / μg L^{-1}
Ni	0.14	0.47
Cu	0.15	0.50
Pb	0.18	0.60
Zn	0.03	0.11
	ICP-MS	
	LOD / μg L^{-1}	LOQ / μg L^{-1}
Ni	0.0022	0.0073
Cu	0.0065	0.0217
Pb	0.0040	0.0133
Zn	0.009	0.031

Finally, other sorbent materials based on IIPs have offered LODs for Ni of 5.0 μg L^{-1} (FAAS detection) [31], 0.3 μg L^{-1} [9] or 0.18 μg L^{-1} [35] when using ETAAS, and 0.16 μg L^{-1} for ICP-OES measurements [40].

3.4.3. Repeatability and accuracy of the method

The repeatability of the over-all procedure was assessed by analyzing a seawater sample eleven times. The IIP-SPE pre-concentration procedure implies a pre-concentration factor of 40 (100ml of seawater sample). The percent relative standard deviation (RSD) values were 4, 7, 8 and 7% for Ni, Cu, Pb and Zn determination by ICP-OES, while RSDs of 6% for Ni and 5% for Cu, Pb and Zn were achieved by using ICP-MS.

Analytical recovery was assessed for three concentration levels, after spiking different aliquots from the same seawater sample with 1.0, 2.0 and 4.0 μg L^{-1} of Ni, Cu, Pb, and with 5, 10 and 20 μg L^{-1} of Zn. Each concentration level (low, medium and high) was tested by triplicate; thus, analytical recoveries (Table 6) are given as mean ± S.D. for three independent measurements.

Table 6. Analytical recovery of the methods (N=3)

	ICP-OES		
	Low[a]	Medium[b]	High[c]
Ni	105 ± 7	103 ± 3	102 ± 2
Cu	95 ± 3	98 ± 3	100 ± 2
Pb	93 ± 2	98 ± 1	104 ± 4
Zn	99 ± 3	99 ± 6	104 ± 5
	ICP-MS		
	Low[a]	Medium[b]	High[c]
Ni	103 ± 2	104 ± 1	100 ± 1
Cu	95 ± 5	103 ± 2	99 ± 1
Pb	95 ± 2	105 ± 1	101 ± 3
Zn	108 ± 2	109 ± 1	101 ± 2

(a) 1.0 μg L^{-1} for Ni, Cu and Pb; 5 μg L^{-1} for Zn

(b) 2.0 μg L^{-1} for Ni, Cu and Pb; 10 μg L^{-1} for Zn

(c) 4.0 μg L^{-1} for Ni, Cu and Pb; 20 μg L^{-1} for Zn

It can be concluded that good analytical recovery (within the 95 – 105% range) are reached for all the target elements and concentration levels.

The analysis of estuarine water (SLEW-3) and lake water (TM-23.3 and TM-24) was performed to assess accuracy. Each CRM was subjected to the IIP-SPE procedure three times using a sample volume of 100mL (pre-concentration factor of 40). Then, determinations were carried out by ICP-OES and ICP-MS. Results, listed in Table 7, reveal good agreement between concentrations found and the certified values for the three certified reference materials. This fact has been proved after applying a t-test (95% confidence range) for comparing the mean values.

Table 7. Analysis of certified reference materials. Each material was analyzed by triplicate.

	ICP-OES					
	SLEW-3		TM-23.3		TM-24	
	Certified / µg L^{-1}	Found / µg L^{-1}	Certified / µg L^{-1}	Found / µg L^{-1}	Certified / µg L^{-1}	Found / µg L^{-1}
Ni	1.2±0.1	1.3±0.1	5.4±0.6	5.7±0.3	3.5±3.0	3.3±0.3
Cu	1.6±0.1	1.4±0.1	9.1±0.6	9.2±1.0	8.0±4.1	7.2±0.5
Pb	0.009±0.001	< LOD	3.2±0.3	3.3±0.6	7.3±2.6	6.9±1.8
Zn	0.20±0.04	0.20±0.02	----[a]		5.4±2.3	5.1±3.0

	ICP-MS			
	SLEW-3		TM-23.3	
	Certified / µg L^{-1}	Found / µg L^{-1}	Certified / µg L^{-1}	Found / µg L^{-1}
Ni	1.2±0.1	1.3±0.04	5.4±0.6	5.8±0.1
Cu	1.6±0.1	1.6±0.02	9.1±0.6	9.6±0.7
Pb	0.009±0.001	< LOD	3.2±0.3	3.5±0.3
Zn	0.20±0.04	0.19±0.02	----[a]	

(a) Not given

3.4.4. Study of the lifetime of the Ni-IIP support

Three different Ni-IIP cartridges were tested in order to know the number of sequential solid phase extraction (loading/elution cycles) that can be performed without losses on pre-concentration efficiency. Experiments were carried out by treating estuarine seawater samples and aqueous standard solutions containing 1.0 µg L^{-1} of Ni, Cu and Pb, and 5.0 µg L^{-1} of Zn. After each five loading/elution cycles with estuarine seawater samples an aqueous standard solution was pre-concentrated and the analytical recovery calculated. Results have shown that Cu, Pb and Zn are not efficiently retained/elute after

around twenty uses (analytical recoveries lower than 95%). However, quantitative analytical recoveries were obtained for Ni up to 40 SPE cycles. It must be said that the lifetime of the polymer is larger for the analyte template (Ni) than for other target elements (Cu, Pb and Zn). Therefore, the Ni-IIP cartridges can be used at least forty times without losing of efficiency of the adsorbent IIP for nickel determination; however, for a multi-element pre-concentration, the IIP can only be used twenty-five times.

4. Conclusions

Ion imprinted polymer particles synthesized from a ternary [Ni(II)-DEM-8-HQ] pre-polymer complex have shown imprinting properties for Ni an also for Cu, Pb and Zn. The synthesized polymeric material has not offered affinity for major elements in seawater samples, such as sodium and potassium, efficiently removing the salt matrix of seawater. Therefore, Ni, Cu, Pb and Zn can be measured against external aqueous calibrations in 2.0M nitric acid. The synthesized IIP can be used at least 25 times without losing the adsorbent properties for the target analytes. In addition, the lifetime of the material can be increased up to 40 SPE cycles if only nickel is determined. Because the large lifetime of the polymer and the low cost for synthesizing it, the Ni-IIP results cost effective when comparing to other adsorbents for SPE of trace metals. The fast kinetics for adsorption/elution is other advantage. Volumes of 100mL of seawater can be treated in 40 min. When using a vacuum manifold station and several samples are simultaneously treated, a sampling frequency of 9 samples per hour is achieved. Finally, the IIP-SPE – ICP-OES/MS method has offered accurate results for the analysis of low salinity samples (lake water) and high salinity samples (estuarine water).

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On line ionic imprinted polymer selective solid-phase extraction of nickel and lead from seawater and inductively coupled plasma – optical emission spectrometry determination

Natalia García–Otero, Carmen Teijeiro–Valiño, Jacobo Otero-Romaní, Elena Peña-Vázquez, Antonio Moreda–Piñeiro, Pilar Bermejo–Barrera

ANALYTICAL AND BIOANALYTICAL CHEMISTRY

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RESUMEN

Durante el siguiente trabajo de investigación se han sintetizados dos polímeros de impronta iónica mediante la técnica de polimerización por precipitación, y se han aplicado para la extracción en fase sólida de níquel (II) y plomo (II) en el agua de mar, mediante un sistema análisis por inyección de flujo acoplado a un Espectrómetro de Emisión Óptica por Plasma Acoplado Inductivamente (FIA-ICP-OES).

Se han optimizado aquellos parámetros que afectan a la extracción de los metales, y se han comparado sus características analíticas mediante la realización de diversos estudios de reactividad cruzada, precisión y exactitud. El polímero de impronta para el plomo ha resultado ser más selectivo que el polímero de niquel, sin embargo, presenta una cinética de adsorción y desorción más lenta (velocidad de carga de 3,0 ml min^{-1} durante 4 min y velocidad de elución de 2,25 ml min^{-1} durante 1 min), lo que implica un factor de enriquecimiento de 5. El polímero de impronta para el níquel presenta un factor de enriquecimiento de 15 (velocidad de carga de 2,25 ml min^{-1}, durante 2 minutos y velocidad de elución de 2,25 ml min^{-1} durante 1 min). Bajo estas condiciones se obtienen ciclos de preconcentración y medición de 5 y 7 minutos para el Ni y Pb, respectivamente, lo que permite analizar 12 muestras para Ni o 8 muestras para Pb por hora.

El límite de detección fue de 0,33 µg l^{-1} para el níquel y 1,88 µg l^{-1} para el plomo, con una precisión del 8% para el níquel (2,37 µg l^{-1}) y del 11% para el plomo (8,38 µg l^{-1}), siendo n = 11. También se evaluó la exactitud de los procedimientos mediante el análisis de dos materiales de referencia (SLEW-3 y TM-24), obteniéndose valores acordes con las concentraciones certificadas. Los materiales poliméricos sintetizados permiten, además, la separación de estos iones de los componentes mayoritarios de la

matriz del agua de mar, pudiendo ser utilizados, por lo menos, 40 veces sin perder las propiedades adsorbentes para los analitos objetivo.

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On line ionic imprinted polymer selective solid-phase extraction of nickel and lead from seawater and inductively coupled plasma – optical emission spectrometry determination

Natalia García–Otero, Carmen Teijeiro–Valiño, Jacobo Otero-Romaní, Elena Peña-Vázquez, Antonio Moreda–Piñeiro, Pilar Bermejo–Barrera

Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain.

Abstract

Nickel (II) and lead (II) ionic imprinted 8-hydroxyquinoleine (8-HQ) polymers were synthesized by a precipitation polymerization technique and were used as selective solid-phase extraction supports for the determination of nickel and lead in seawater by flow injection solid phase extraction on line inductively coupled plasma – optical emission spectrometry (ICP-OES). An optimum loading flow rate of 2.25 mL min^{-1} for 2 min and an elution flow rate of 2.25 mL min^{-1} for 1 min gave an enrichment factor of 15 for nickel. However, low dynamic capacity and/or kinetic for adsorption and desorption was found for Pb-IIP and flow rate of 3.00 mL min^{-1} for 4 min loading and flow rate of 2.25 mL min^{-1} for 1 min elution gave a enrichment factor of 5. The limit of detection was 0.33 μg L^{-1} for nickel and 1.88 μg L^{-1} for lead, with precision (n = 11) of 8% (2.37 μg Ni L^{-1}) for nickel and 11% (8.38 μg Pb L^{-1}) for lead. Accuracy was also assessed by analyzing SLEW-3 (estuarine water) and TM-24 (lake water) certified reference materials, and the determined values were in good agreement with the certified concentrations.

Keywords

On line solid-phase extraction, ionic imprinted polymer, nickel, lead, seawater, inductively coupled plasma – optical emission spectrometry

1. Introduction

Nickel and lead have been long recognized to be toxic metals for marine ecosystems. Both elements enter to the aquatic environment from the dissolution of rocks and soils, from atmospheric fallout, but mainly from industrial processes and water disposal [1,2]. This last fact is especially important for estuarine systems because they are near the different industries and villages located along the coast. In addition, estuaries are also used for different aquaculture activities, such as mollusk production, and the levels of heavy elements in estuarine water are limited by several legislation directives [3]. Therefore, continuous development of reliable analytical methods to assess these elements in complex samples is of interest.

Heavy metals are present at very low concentrations in estuarine/seawater, while matrix concomitants such as chloride and alkaline elements (sodium, potassium, etc.) occur at very high levels [1]. Therefore, pre–concentration/separation methods combined with sensitive and selective detectors are needed to perform nickel and lead determination in seawater. Among the pre–concentration procedures for trace elements, solid phase extraction techniques (SPE) are quite popular because they offer a number of important advantages over other pre–concentration methods. One of the main benefits is that adsorbent material can be packed into mini–columns or cartridges which provide sufficient exchange/adsorption capacity for many applications and ease of automation [4]. Most of the SPE procedures use ion exchange/chelating resins which pose the

disadvantage of a previous derivatization stage when pre–concentrating inorganic species such as trace metals. This is because only uncharged (non polar) species can interact with most of the adsorbent materials [5]. For example, when using high purity C18 (silica-based materials) as an adsorbent material for lead and/or nickel, the use of a chelating reagent such as 8-HQ is needed in order to obtain uncharged complexes – adsorbent interactions [6-8]. In addition, another disadvantage of such materials for metal pre-concentration is the lack of selectivity which generates matrix effect and the use of the standard addition technique for carrying out the determinations [8].

High selectivity for SPE procedures can be obtained by using solid supports synthesized by molecular imprinting polymerization or by ionic imprinting polymerization. These materials can be prepared (synthesized) using inexpensive reagents and with a general knowledge of synthesis. For either molecular imprinted polymer (MIP) or ionic imprinted polymer (IIP), a monomer is polymerized in the presence of a template (a molecule, an ion or a metal-complex) and an excess of a cross-linker, and after the template leaching, the MIP (IIP) is capable of recognizing and rebinding the target analyte (the template) with high selectivity and affinity [9,10]. Among the different approaches for the synthesis of IIPs [11,12], the trapping of non-vinylated chelating ligand (complexing agent) via imprinting of binary/ternary mixed ligand complexes of metal ions with non-vinylated chelating agent and a vinyl ligand is a popular used method [12]. The complexing agent is trapped into the polymeric material, showing affinity for the ion (template) which was used for synthesis. Complexing agents such as dimethylglyoxime [13], diazoaminobenzene [14], cetyltrimethyl ammonium bromide [15] and derivates of quinoline (5,7-dichloroquinoline-8-ol) [16-25] have been successfully applied, mainly for lanthanides, although some application can be found in literature for transition metals such as nickel [24,25].

All IIPs-based SPE procedures commented above are off-line processes which are time consuming methodologies. If the IIP materials can offer high dynamic capacity and/or kinetic for adsorption and desorption, pre-concentration can be carried out without reaching the thermodynamic equilibrium in short times, being suitable for on-line pre-concentration approaches. Some examples of on-line SPE using ionic imprinted sorbents can be found in literature for both lanthanides [26] and also transition metals such as copper [27]. Therefore, one of the goals of the current work is evaluating the possibilities of two synthesized IIPs for the on-line pre–concentration/separation of traces of nickel and lead from seawater samples. Synthesized IIP adsorbent material was packed into short cartridges which were incorporated in a 6–way valve of an automatic flow injection manifold combined with inductively coupled plasma–optical emission spectrometry detection. Inexpensive reagents such as 8-HQ, as a non-vinylated reagent; 2-(diethylamino) ethyl methacrylate (DEM), as a monomer; and divinylbenzene-80 (DVB), as a cross-linker were used for synthesis by the precipitation polymerization technique.

2. Experimental

2.1. Apparatus

An Optima 3300 DV inductively coupled plasma atomic emission spectrometer (Perkin Elmer, Norwalk, CT, USA) equipped with an autosampler AS 91 (Perkin Elmer) and a Gem-Cone cross-flow nebulizer type (Perkin Elmer) was used for lead and nickel determinations. Detection wavelengths were 231.604 and 224.688nm for Ni and Pb, respectively. Radiofrequency power was 1300W and argon flow rates were 15.0 L min^{-1} for plasma, 0.5 L min^{-1} for the auxiliary gas and 0.8 L min^{-1} for the nebulization. A

Rheodyne (model 5020) six-port low-pressure flow-injection valve (Rheodyne, Cotati, CA, USA) with PTFE tubes (0.8mm i.d.) was connected to a peristaltic pump (Perkin Elmer) for direct analyte introduction to the nebulizer-plasma torch system, and to a Miniplus 3 peristaltic pump (Gilson, Middleton, WI, USA) for sample load and eluting solution pumping. Solvent flexible tubing red/red, 1.14mm (SCP Science, Montreal, Canada) were used for sample loading and eluting solution pumping through the cartridges, while solvent flexible tubing black/black, 0.76mm, and white/black, 1.52mm (SCP Science) were used for eluate introduction into the plasma torch and for waste removal, respectively. Synthesized IIP material (300 mg) was placed into empty Sep–Pak® cartridges (plus short) from Waters (Milford, MA, USA) between 20μm porosity Teflon frits (Supelco, Bellefonte, PA, USA). An incubation camera model Boxcult from Selecta (Barcelona, Spain) with controlled temperature coupled with low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. An ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Chemicals were ultrapure grade, using ultrapure water, resistance 18 MΩ cm (Millipore Co., Bedford, MA, USA). Stock nickel and lead standard solutions (1.000 g L^{-1}) were supplied by Merck (Darmstadt, Germany). Analytical grade nickel chloride ($NiCl_2.6H_2O$) and lead nitrate ($Pb(NO_3)_2$), used as templates, were purchased from Panreac (Barcelona, Spain). High purity nitric acid 69% was from Panreac, while high purity ammonia, ammonium chloride, and analytical grade 8-hydroxyquinoline were purchased from Merck. HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). 2-(diethylamino) ethyl methacrylate (DEM), used as a monomer, and divinylbenzene-80 (DVB), used as a cross-linker, were from Sigma-

Aldrich (Steinheim, Switzerland). DVB was treated in order to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing approximately 0.5g of neutral alumina (Sigma-Aldrich). AIBN (2,2′-azobisisobutyronitrile) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at -20°C after dissolving the reagent in methanol (Merck) at 50–60°C. After purification, this reagent was stored at 4°C. Estuarine seawater (SLEW–3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM–24) certified reference material was purchased from the National Water Research Institute of Canada.

2.3. Seawater samples collection.

Seawater samples (1L) were collected from the Ría de Muros-Noia estuary (north-western Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were filtered through 0.45μm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then and stored at low temperature until used.

2.4. Synthesis of nickel and lead ionic imprinted polymers

Table 1 lists the amounts (weights or volumes) of template (nickel chloride or lead nitrate), monomer (DEM), complexing agent (8-HQ), cross-linker (DVB) and free radical initiator (AIBN) used in the synthesis of Ni-IIP [25] and Pb-IIP. The general procedure was as follows: first, the template is mixed with the monomer and the complexing agent into 15mL glass tubes before adding 12.5mL of porogen (3:1 acetonitrile:toluene). The mixture is then stirred for 5 minutes and filtered if necessary. Finally, the cross-linker and the initiator are added to the mixture (volumes or weights given in Table 1) and the glass tubes are purged with N_2 and immediately sealed just before thermal induction of the precipitation polymerization.

Table 1. Molar Template/Monomer/Ligand ratio and masses and volume of the different reagents involved into the polymerization process.

	Template	Monomer (DEM)	Ligand (8-HQ)	Cross linker (DVB)	Initiator (AIBN)	Molar ratio (Template/Monomer/Ligand)	Mass of polymer[b]
Ni-IIP	0.0334g[a]	56.5µL	0.0820g	500µL	0.0420g	1/2/4	0.3121 g (62%)
Pb-IIP	0.0355g[b]	60.0µL	0.0842g	500µL	0.0413g	1/2/4	0.3080 g (62%)

(a) $NiCl_2 \cdot 6H_2O$

(b) $Pb(NO_3)_2$

The temperature was increased from room temperature to 60ºC over 2h, and then maintained at 60ºC for a further 24h under a low stirring rate (33rpm). The polymer particles obtained are then vacuum filtered, washed with acetonitrile and oven-dried overnight at 40ºC. It can be seen from Table 1 that the polymers were synthesized in presence of a double amount of 8-HQ (complexing agent) with respect to DEM (monomer). This gives a Template/DEM/8-HQ molar ratio of 1/2/4. The masses of polymer obtained after polymerization were around 0.300g and the efficiency of the polymerization process, taking into account a theoretical amount of synthesized polymer of 0.5g, was around 60%. Blank polymer particles (non imprinted polymer, NIP) were also prepared in the same way as IIP, but without the template. The NIP was then subjected to the same washing pre-treatment as described above.

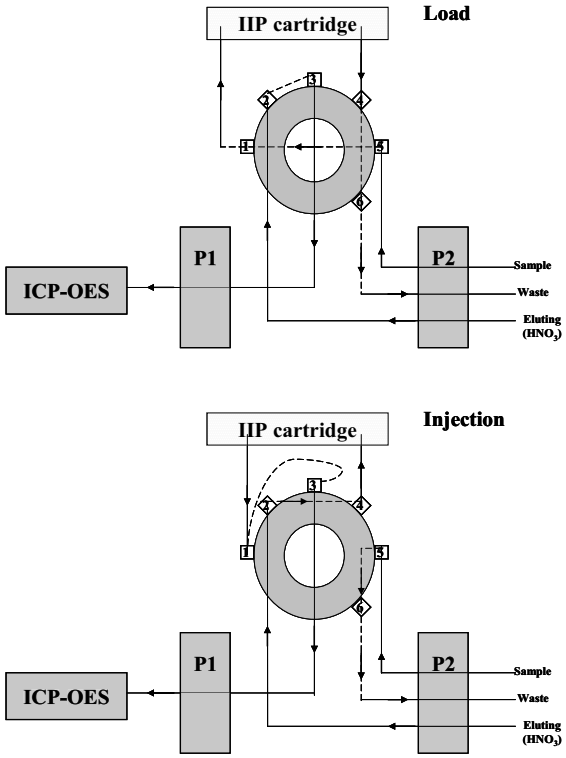
2.5. IIP cartridges preparation.

Empty Sep–Pak® cartridges were filled with 0.30 g of IIP or NIP adsorbents. The IIP or NIP material was between two Teflon frits. Once prepared, the IIP cartridge was incorporated in the 6–way valve and the template (Ni(II) or Pb(II) ions) were removed from the polymer particles by extensive washing with 2.0M nitric acid. An efficient template removal from IIP particles was achieved after passing 50mL of the washing solution. This was verified by ICP-OES measurements of nickel or lead in the washing (eluting) solutions from the cartridges (6-way valve in injection position, according to Figure 1).

2.6. On line pre–concentration – ICP-OES determination.

Under optimum conditions, the pH of the acidified seawater samples was adjusted to 9.0 for nickel pre-concentration or to 8.5 for lead pre-concentration by adding low amounts of 6.0M ammonia solution; or preparing aqueous standard in 0.1M/0.1M NH_3/NH_4Cl buffer solution at pH 9.0 (nickel pre-concentration) or 8.5 (lead pre-concentration).

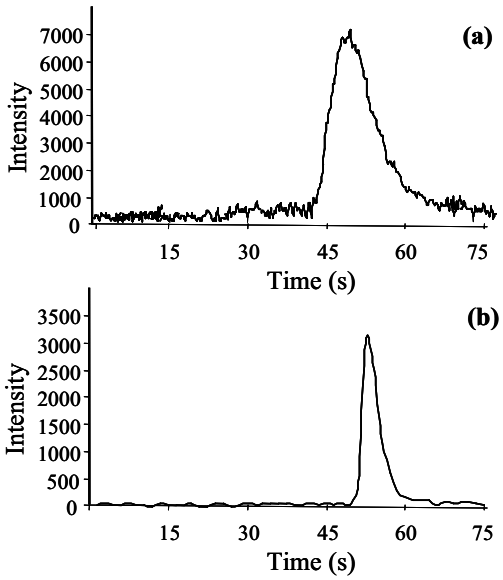
Figure 1. Schematic diagram of the flow injection manifolds used for the pre–concentration–ICP-OES determination.



A similar procedure was adopted when testing other pHs (6.0, 8.0, 8.5 and 9.0) as shown in section 3.4. A diagram showing the load (adsorption) and elution steps in the on line nickel or lead IIP-SPE is given in Figure 1.In the pre-concentration (loading) step, the seawater sample (at pH of 9.0 for nickel or at pH of 8.5 for lead) is drawn through the cartridge containing the IIP material by means of at flow rate of 2.25 mL min^{-1} for 120s (nickel pre-concentration) or 3.00 mL min^{-1} for 240s (lead pre-concentration). In the meantime, the eluting solution (3.0M nitric acid) is pumped by pump 2 and pump 1 through the detector (Figure 1a). In the elution step (Figure 1b), the

eluting solution is propelled through the column in the opposite direction to load at a flow rate of 2.25 mL min^{-1} for 60s, time enough to elute the analytes completely, as it is shown in Figure 2 (FIAgrams for nickel (a) and lead (b) measurements). The eluting solution is directly introduced to the detector and the nickel or lead emission signals are continuously monitored. Previous to the loading step, the IIP material is conditioned by passing a 0.1M/0.1M NH_3/NH_4Cl buffer solution at pH 9.0 (nickel pre-concentration) or 8.5 (lead pre-concentration) through the cartridge at a flow rate of 2.25 mL min^{-1} for 60s (optimum conditions); or by passing 0.1M/0.1M NH_3/NH_4Cl buffer solution at other pHs for optimization studies (section 3.4.). Similarly, the same buffer solution at the same flow rate and time conditions is passed through the cartridges just before the eluting step in order to remove adsorbed matrix concomitants from the IIP material.

Figure 2. FIAgram for a seawater sample spiked with 20 µg L^{-1} of Ni(II) (a) or 20 µg L^{-1} of Pb(II) (b) .



Determinations were performed by using aqueous standard solutions in 0.1M/0.1M NH_3/NH_4Cl at pH 9.0 (nickel) or 8.5 (lead) covering nickel concentrations within the 0–50 μg L^{-1} range and lead concentrations until 100 μg L^{-1}.

3. Results and discussion

3.1. Preliminary evaluation of IIPs under static conditions

To measure the static adsorption capacity, 300 mg of imprinted (Ni-IIP and Pb-IIP) sorbents were saturated (flow rate at 10 mL min^{-1}) with several 3.0 mL aliquots of 200 μg mL^{-1} Ni(II) or 100 μg mL^{-1} Pb(II) solutions at pH 8.5 [8,25] and then, the nickel or lead content in the eluates were directly measured by ICP-OES. The static adsorption capacity of the Ni-IIP and Pb-IIP were 0.023 and 0.0015 mmol g^{-1}, respectively, which implies calculated retention capacities of 11% and 7% for Ni-IIP and Pb-IIP, respectively. These calculated retention capacities are around 10% of the theoretical retention capacity, which agrees with reported retention capacities for MIPs [28].

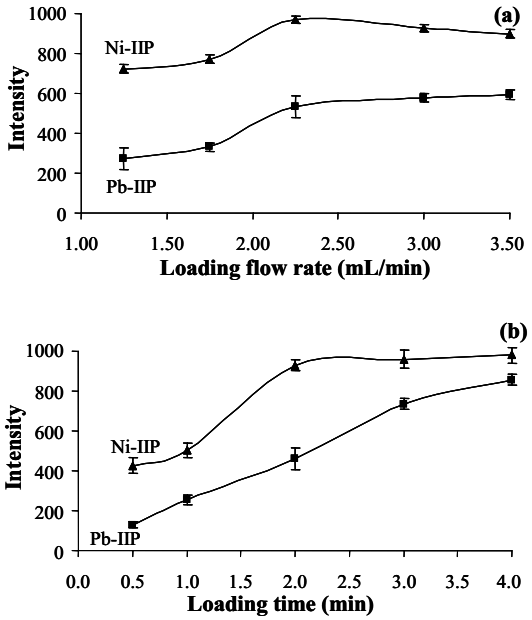
Similarly, the breakthrough volume was determined under static conditions. Different volumes (25, 100 and 200mL) of aqueous Ni(II) or Pb(II) standard solution, both at 50 μg L^{-1}, were adjusted at a pH 8.5 [8,25], and passed through cartridges packed with 300 mg of both IIPs at a flow rate of 10 mL min^{-1}. The retained nickel or lead were then eluted with 2.5 mL of 2.0 M nitric acid and determined by ICP-OES. It was observed that large volumes of loading solutions can be used for both IIPs containing 300 mg of the polymeric material and the breakthrough volume is not reached even after loading with 200 mL of sample solutions, showing an enhancement factor of 80 under static conditions.

3.2. Optimization of loading conditions (dynamic adsorption conditions).

Loading flow rate and loading time were first studied under fixed elution conditions: an elution flow rate of 3.0 mL min^{-1}, a nitric acid concentration in the eluting solution at 2.0M, and an elution time of 1.0 min. Aqueous standard solutions containing Ni(II) or Pb(II) at 50.0 µg L^{-1} or a seawater sample spiked with Ni(II) or Pb(II) at 20.0 µg L^{-1} were used as samples. Ni(II) or Pb(II) aqueous standards were prepared in a 0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 8.0. The pH of the spiked seawater samples, acidified with nitric acid added, was increased to 8.0 by adding small volumes of 5.0M NH_3. This pH was selected in based on previous off line experiments for metal-8-HQ complexes and C18 support materials and also for IIPs for Ni(II) pre-concentration [8,25]. A washing stage just before the elution stage was applied by pumping a 0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 8.0 at a flow rate of 3.0 mL min^{-1} for 1.0 min.

Figure 3 (a-b) shows the average peak height (three replicates) for spiked seawater samples when varying the loading flow rate from 1.25 to 3.5 mL min^{-1} and for loading times within the 0.5 to 4.0 min range. It has been found that nickel intensity was slowly decreased when increasing the load flow rate from 2.25 to 3.50 mL min^{-1}, while high lead signals were obtained for high load flow rates (Figure 3a). Similarly, the highest nickel signals were obtained for load times above to 2.0 min. However, a higher load time is need to obtain the highest lead signal (Figure 3b). These results imply a low dynamic capacity and/or kinetic for adsorption/desorption for Pb-IIP. A load flow rate of 3.00 mL min^{-1} for a load time of 4.0 min are needed for pre-concentrating lead, while a load flow rate of 2.25 mL min^{-1} for a load time of 2.0 min is enough for the use of the Ni-IIP. Similar results were obtained when using Ni(II) or Pb(II) buffered aqueous standard solutions. Therefore, under these loading conditions, a volume of 14 mL is passed through the cartridge containing Pb-IIP but only 4.5 mL are required for Ni-IIP.

Figure 3. Effect of the loading flow rate (a) and the load time (b) on the nickel and lead emission intensities (peak height).

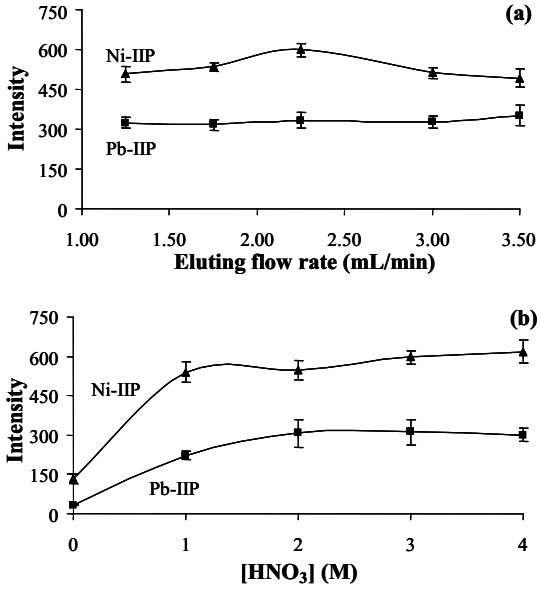


3.3. Optimization of eluting conditions (dynamic adsorption conditions).

By fixing loading conditions at 2.25 mL min^{-1} for 2.0 min (Ni-IIP) and at 3.00 mL min^{-1} for 4.0 min (Pb-IIP), the eluting flow rate was studied from 1.25 to 3.5 mL min^{-1}. A nitric acid solution at 2.0M was used as eluting solvent and also either Ni(II) or Pb(II) aqueous standard solutions and spiked seawater samples were buffered at pH 8.0. Figure 4a shows the nickel signal decreases when using eluting an flow rate higher than 2.25 mL min^{-1}, while the lead signal remains constant within the eluting flow rate studied.

By using an eluting flow rate of 2.25 mL min^{-1}, the effect of the nitric acid concentration on the nickel and lead signals is plotted in Figure 4b. It can be seen that a nitric acid concentration of 3.0 M was sufficient to elute both metals from the polymer particles.

Figure 4. Effect of the eluting flow rate (a) and the nitric acid concentration (b) on the nickel and lead emission intensities (peak height).

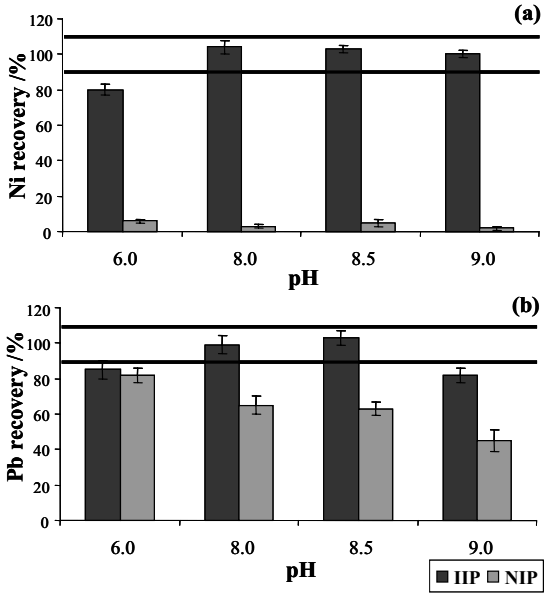


Similar results have been obtained for the use of Ni(II) or Pb(II) buffered aqueous standard solutions. In addition, the effect of the time for the washing stage between the loading and injection steps were not found to be significant, and a washing time of 1.0 min was finally selected.

3.4. Effect of pH. Imprinting effect.

The effect of the pH on the Ni(II) or Pb(II) retention was studied for cartridges filled with synthesized Ni-IIP and Pb-IIP materials as well as for Ni- and Pb- non imprinted polymers (Ni-NIP and Pb-NIP). Different buffered aqueous standards (at the studied pHs) covering Ni or Pb concentrations between 0 and 50 μg L^{-1} and an unspiked seawater sample and seawater spiked with 20 μg L^{-1} of Ni(II) or Pb(II) were used for the experiment. Results as analytical recoveries are plotted in Figure 5a for Ni-IIP and Ni-NIP, and in Figure 5b for Pb-IIP and Pb-NIP.

Figure 5. Effect of pH on the analytical recovery for nickel (a) and lead (b) when using IIPs and NIPs.



It can be seen that Ni is quantitatively retained when using pHs between 8.0 and 9.0 (nickel recoveries from 104 ± 4% to 100 ± 2%), while nickel recoveries for the use of the NIP are lower than 6% for all pHs. This means that interactions between nickel ions and the polymer particles are specific interactions (imprinting effect) even when working at a pH of 6.0 (non quantitative retention). Therefore, it can be said that imprinted cavities for Ni(II) have been produced during the synthesis of Ni-IIP.

Quantitative lead recoveries (Figure 5b) were only reached when working at pHs of 8.0 and 8.5 (lead recoveries of 99 ± 5% and 103 ± 4%, respectively), obtaining lead recoveries of around 80% when using pHs of 6.0 and 9.0. However, lead recoveries close to 60% were obtained for the use of Pb-NIP at pHs of 8.0 and 8.5. This means that

interactions between lead ions and the polymer particles can be attributed to specific (imprinting) and non specific (adsorption) interactions. Although a high percentage extraction (around 60% in Figure 5b) was obtained for the use of Pb-NIP, the Pb-IIP offers imprinting capabilities for Pb(II) ions because the difference between the percentage extraction of the Pb-IIP and Pb-NIP is close to 40%. In this sense, other IIPs reported in literature have offered differences lower than 30% [16,23] or even lower than 20% [17]. It must be noticed that the Pb-IIP does not work as an imprinted polymer when working at pH 6.0 since lead recoveries close to 80% for both Ni-IIP and Pb-NIP are obtained.

From the results obtained, a pH of 9.0 was finally chosen for nickel pre-concentration, while a pH of 8.5 was preferred for lead.

3.5. Effect of major components from seawater

A set of experiments was carried out to observe interactions between the polymeric materials and the major metals present in seawater (Na, K, Ca and Mg). A seawater sample was subjected seven times to the proposed procedure and the concentrations of major ions were determined by ICP-OES. After pre-concentration, concentrations lower than 20 mg L^{-1} were determined for Na, K, Mg and Ca, which are very low concentration taking into account the concentration of such elements in seawater, around 11490, 399, 1293 and 413 mg L^{-1}, for Na, K, Mg and Ca, respectively [1]. Therefore, it can be concluded that salt matrix is efficiently removed for the use of either Ni-IIP or Pb-IIP.

3.6. Cross-reactivity among nickel/lead and other trace elements.

To evaluate the cross-reactivity among nickel or lead and other transition metals (Cd, Cu, Cr, Fe, Mn, Sn, V, and Zn) aqueous solution containing 50 μg L^{-1} of the target element (nickel(II) or lead(II)) and the other trace metals (also at 50 μg L^{-1}) were passed

through the Ni-IIP (and Ni-NIP), and also Pb-IIP (and Pb-NIP) by triplicate after fixing the pH at 9.0 (Ni-IIP and Ni-NIP) or 8.5 (Pb-IIP and Pb-NIP). Results, expressed as percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$ or $S_{Pb/M}$), are listed in Table 2 and Table 3 for Ni-IIP/NIP and Pb-IIP/NIP, respectively. From these results, it can be stated that Pb-IIP has shown good selectivity for lead over other transition metals, and imprinting properties are only observed for lead. In should be mentioned that Cr is quantitatively retained in Pb-IIP, but this retention is unspecific (similar percent extraction is obtained for Cr when using Pb-NIP).

Table 2. Percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$) for Ni-IIP and Ni-NIP.

	IIP			NIP		
Element	E (%)[a]	D[b]	$S_{Ni/M}$[c]	E (%)[a]	D[b]	$S_{Ni/M}$[c]
Ni	99	99	-	1	0.01	-
Cd	76	3	33	13	0.2	0.05
Cu	93	13	8	39	0.6	0.02
Cr	98	49	2	99	99	0.0001
Fe	82	5	20	84	5	0.002
Mn	20	0.2	495	12	0.1	0.1
Pb	95	19	5	34	0.5	0.02
Sn	45	0.8	124	78	3	0.003
V	90	9	11	75	3	0.003
Zn	90	9	11	37	0.6	0.02

(a) E (%) = $(A_2/A_T)\times100$; (b) D = (A_2/A_1); (c) $S_{Ni/M} = D_{Ni}/D_M$.

A_1 =Amount of metal ion in aqueous solution at equilibrium; A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium. A_T = Total amount of metal ion used in extraction. D_{Ni} = Distribution ratio for Ni; D_M = Distribution ratio for M (M = Cd, Cu, Cr, Fe, Mn, Pb, Sn, V and Zn).

Table 3. Percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Pb/M}$) for Pb-IIP and Pb-NIP.

	IIP			NIP		
Element	E (%)[a]	D[b]	$S_{Ni/M}$[c]	E (%)[a]	D[b]	$S_{Ni/M}$[c]
Pb	99	99	-	55	1	-
Cd	76	3	33	22	0.3	3
Cu	80	4	25	76	3	0.3
Cr	92	12	8	95	19	0.05
Fe	85	6	16	81	4	0.2
Mn	75	3	33	70	2	0.5
Ni	85	6	16	75	3	0.3
Sn	83	5	20	80	4	0.2
V	80	4	25	75	3	0.3
Zn	57	1	76	40	0.7	1

(a) E (%) = $(A_2/A_T)\times100$; (b) D = (A_2/A_1); (c) $S_{Pb/M} = D_{Pb}/D_M$.

A_1 =Amount of metal ion in aqueous solution at equilibrium; A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium. A_T = Total amount of metal ion used in extraction. D_{Pb} = Distribution ratio for Pb; D_M = Distribution ratio for M (M = Cd, Cu, Cr, Fe, Mn, Ni, Sn, V and Zn).

However, Ni-IIP has shown certain imprinting effect for Cu, Pb and Zn, and also non specific interactions for Cr (Table 3). The relatively low selectivity for Ni-IIP can be attributed to the fact that the hydroxyquinoline ring (8-HQ as a ligand) is not linked to the polymeric chains forming rigid imprinting cavities. In this case, 8-HQ is trapped into the polymeric matrix after polimerization [25] and the imprinting cavities appear to offer certain “flexibility”. Because the multi-element capabilities of Ni-IIP, this polymer can be used for the simultaneous pre-concentration of Ni moreover Cu, Pb and Zn from seawater samples.

3.7. Analytical performances.

Five different buffered aqueous calibrations and standard addition graphs (pH 9.0 for Ni or 8.5 for Pb), both covering nickel concentrations from 0 to 50 μg L^{-1} or lead concentrations from 0 to 100 μg L^{-1} were obtained. Mean slopes of 126.2 ± 9.161 L μg^{-1} and 109.5 ± 10.73 L μg^{-1} were obtained for Ni aqueous standard and Ni standard addition graphs, respectively; while a mean value of 49.1 ± 2.44 L μg^{-1} was obtained for Pb aqueous standards and a mean value of 48.2 ± 1.72 L μg^{-1} was reached for Pb standard addition.

After application of Cochran'C and Bartlett's tests at a 95.0% significant level for comparing variances and an ANOVA test for comparison of means, also at a 95.0% significant level, it could be concluded that slopes of aqueous calibration and standard addition graphs for Ni and Pb are statistically comparable (95.0%) and therefore matrix effect is not observed for both determinations.

In order to obtain the enrichment factor as a ratio of slopes from an aqueous calibration through the cartridges (internal calibration) and from an aqueous calibration without pre-concentration (external calibration by direct aspiration of standard solutions), three different aqueous calibrations covering Ni and Pb concentrations between 0 and 100 μg L^{-1} were obtained. A mean slope of 8.50 ± 0.955 L μg^{-1} was calculated for Ni determination, which means an enrichment factor of 14.8. Similarly, a mean slope of 9.50 ± 1.23 L μg^{-1} was obtained for the Pb external aqueous calibration, meaning an enrichment factor of 5. The limit of detection (3SD/m) and the limit of quantification (10SD/m), where S.D. is the standard deviation of eleven measurements of a blank and m is the mean slope of the aqueous calibration graph, were 0.33 and 1.10 μg L^{-1}, respectively for nickel. Values of 1.88 and 6.27 μg L^{-1} for LOD and LOQ, respectively, were obtained for lead.

Table 4. Analysis of certified reference materials. Each material was analyzed by triplicate.

	SLEW-3 (estuarine water)		TM-24 (lake water)	
	Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})
Ni	1.23 ± 0.07	1.12 ± 0.08	3.5 ± 3.0	2.5 ± 0.19
Pb	0.0090 ± 0.0014	< 1.88	7.3 ± 2.6	6.4 ± 0.38

The repeatability of the over–all procedure was assessed by analyzing a surface seawater sample eleven times by the on-line IIP-SPE-ICP-OES method. The RSD value was 8% for nickel determination (nickel concentration of 2.37 ± 0.181 μg L^{-1}) and 11% for lead (lead concentration of 8.38 ± 0.907 μg L^{-1}).

The accuracy of the method was assessed by analyzing two certified reference materials (CRMs): TM-24 (lake water) and SLEW-3 (estuarine water). Each certified reference material was subjected three times to the optimized on-line pre-concentration ICP-OES procedure. Table 4 lists the found nickel and lead concentrations in both CRMs. It can be seen that nickel and lead levels were within the certified concentration ranges, although results appear to show a small negative bias because both lead and nickel values found are on the lower side of the certification range.

3.8. Study of the lifetime of the Ni-IIP and Pb-IIP SPE supports

To know the lifetime of both IIPs two Ni-IIP and two Pb-IIP cartridges were tested in order to know the number of sequential loading/elution cycles that can be performed without losses on pre-concentration efficiency. Experiments were carried out by treating estuarine seawater samples (five different SPE cycles) followed by an aqueous standard solution containing 50 μg L^{-1} of Ni or 50 μg L^{-1} of Pb. Analytical recovery was assessed

after pre-concentrating the Ni or Pb aqueous standard solution. Results have shown that Ni is efficiently retained/elute in Ni-IIP at least up to forty uses (analytical recoveries higher than 95%). For the following ten SPE cycles, Ni analytical recovery decreased (lower than 95%). However, quantitative analytical recoveries were obtained for Pb up to 50 SPE cycles when using Pb-IIP. Therefore, both IIPs can be used at least forty times without losing of retention efficiency.

4. Conclusions

A simple and cheap procedure for synthesis of ionic imprinted polymers for nickel (II) and lead (II) ions based on trapping a complexing agent (8-HQ) into the polymeric matrix was developed by the precipitation polymerization technique. The IIP materials offer selectivity (imprinting effect) for the template ions (Ni(II) or Pb(II)) and they allow a successful separation of these ions from the concomitant seawater matrix components. The synthesized IIP for Ni exhibits better imprinting properties as well as a fast adsorption and desorption kinetic, which implies an enrichment factor of 15. However, synthesized IIP for Pb exhibits worse imprinting characteristics than those offered by Ni-IIP, and an enrichment factor of 5 is achieved for lead. Since the complexing agent is trapped into the polymeric matrix, a previous derivatization stage by the metal complexation is not needed and only a pH adjustment is required before pre-concentration. In addition, the synthesized IIPs can be used at least 40 times without losing the adsorbent properties for the target analytes. Because the large lifetime of the polymer and the low cost for synthesizing it, the Ni-IIP results cost effective when comparing to other adsorbents for SPE of trace metals. The incorporation of a cartridge filled with the IIP materials in a 6–way valve of a flow injection manifold allows an automated pre–concentration and determination of nickel and lead in seawater by ICP-

OES. Pre-concentration and measurement cycles of 5.0 and 7.0 min have been obtained for Ni and Pb, respectively, which implies the analysis of 12 (Ni determinations) or 8 (Pb determinations) samples per hour. These times are short enough as those offered by conventional off-line pre-concentration SPE procedures

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Ionic imprinted polymer for nickel recognition by using the bi-functionalized 5-vinyl-8-hydroxyquinoline as a monomer: Application as a new solid phase extraction support

Jacobo Otero-Romaní, Antonio Moreda-Piñeiro, Pilar Bermejo-Barrera, Antonio Martin-Esteban

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RESUMEN

Tal y como ha quedado demostrado en nuestros trabajos anteriores, el polímero de impronta iónica preparado a partir de un complejo de prepolimerización formado por Ni(II), 2-dietilamino etil metacrilato (DEM) y 8-hidroxiquinolina (8-HQ) es capaz de reconocer no solo el analito plantilla (Ni (II)) sino también el Cu (II), el Pb (II) y el Zn (II), con recuperaciones analíticas del 100% para todos estos elementos. Este fenómeno se atribuye al hecho de que el agente complejante (la 8-HQ) no posee grupos vinilo polimerizables. Por lo tanto, aunque se encuentra atrapado en la matriz del polímero, no está vinculado químicamente a las cadenas poliméricas, existiendo la posibilidad de que interaccione con otros compuestos iónicos presentes en las muestras acuosas. La disposición de las moléculas de 8-HQ en la matriz polimérica también parece un factor relevante de cara a que se produzca la retención de dichos elementos, pues también ha sido demostrado que el polímero blanco (NIP) no muestra afinidad por ninguno de ellos, probablemente porque los grupos funcionales de los monómeros y el agente complejante no se encuentran dispuestos en la posición geométrica adecuada. Esto se debe, probablemente, a la inexistencia de un complejo de prepolimerización en el cual el monómero y el agente complejante se puedan distribuir adecuadamente alrededor del analito plantilla.

En el siguiente trabajo de investigación se ha sintetizado un nuevo polímero de impronta iónica para la preconcentración de níquel en el agua de mar. El polímero preparado es idéntico al mencionado anteriormente, con la diferencia de que la 8-HQ ha sido sustituida por la 5-vinil-8-hidroxiquinolina (5-VHQ), un derivado de dicho agente complejante que posee un grupo vinilo polimerizable en la posición 5.

Los resultados obtenidos demuestran que el nuevo polímero ofrece una mayor selectividad para el analito plantilla (níquel (II)) de la que ofrece el polímero sintetizado

en presencia de 8-HQ. Este resultado se atribuye al hecho de que el ligando vinilado se encuentra químicamente vinculado a las cadenas poliméricas originando cavidades de impresión "rígidas" en las que los grupos funcionales del compuesto se encuentran inmovilizados.

El polímero sintetizado ha sido utilizado para la extracción en fase sólida de níquel (II) en el agua de mar, empleando el ICP-OES como técnica analítica de detección. Todas las variables que afectan el proceso de SPE, tales como el pH de la muestra, la velocidad de carga y elución, y la concentración y el volumen de la disolución de elución, fueron evaluadas. El procedimiento optimizado consiste en hacer pasar una muestra de agua de mar basificada (pH 9,0 ± 0,1) a través de un cartucho de SPE que contiene 300 mg del polímero, a una velocidad de 3,0 ml min^{-1}. La elución se realizó mediante el paso de 2,5 ml de ácido nítrico 2,0 M a una velocidad de 1,5 ml min^{-1}. Cuando trabajamos con un volumen de muestra de 100 ml de agua de mar se obtiene un factor de preconcentración de 40, dando como resultado un límite de detección de 0,26 μg l^{-1}. La exactitud del método fué evaluada mediante la realización de un estudio de recuperación analítica y mediante el análisis de dos materiales de referencia certificados (SLEW-3 y TM-23.3). La precisión del método se determinó a través de un estudio de reproducibilidad en el que el coeficiente de variación de una muestra (0,93 μg l^{-1}, n=11) resulto ser del 3%. Además, aparte de la selectividad frente a otros metales de transición, el nuevo polímero de impresión iónica tampoco presenta afinidad por los elementos mayoritarios del agua de mar (Na^{+}, K^{+}, Mg^{2+} y Ca^{2+}), lo que permite la determinación de Ni mediante el empleo de una sencilla recta de calibrado acuosa.

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Ionic imprinted polymer for nickel recognition by using the bi-functionalized 5-vinyl-8-hydroxyquinoline as a monomer: Application as a new solid phase extraction support

Jacobo Otero-Romaní[1], Antonio Moreda-Piñeiro[1], Pilar Bermejo-Barrera[1], Antonio Martin-Esteban[2]

(1) Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain. (2) Department of Environment. National Institute for Agriculture and Food Research (INIA). Carretera de A Coruña, Km 7,5. 28040 – Madrid. Spain.

Summary

A new ionic imprinted polymer (IIP) for Ni(II) recognition/pre-concentration was prepared via precipitation polymerization using 2-(diethylamino) ethyl methacrylate (DEM) and divinylbenzene (DVB) as a crosslinking agent in the presence of nickel(II) and 5-vynil-8-hydroxyquinoline (5-VHQ) as a bi-functionalized ligand. An important increase on the selectivity of the synthesised IIP for nickel(II) ions was obtained when comparing to the use of 8-hydroxyquinoline (8-HQ) as a ligand. The synthesised IIP was used as a new support for solid phase extraction (SPE) of nickel(II) from seawater before inductively coupled plasma – optical emission spectrometry (ICP-OES) detection. Variables affecting the SPE process, such as pH, load and elution flow rates and concentration and volume of the eluting solution, were fully evaluated. The optimised procedure consists of a sample loading (100mL of seawater at a pH of 9.0±0.1) through IIP-SPE cartridges containing 300 mg of the synthesised IIP at a flow rate of 3.0 mL min^{-1}. Elution was performed by passing 2.5mL of 2.0M nitric acid at a

flow rate of 1.5 mL min^{-1}, which gave a pre-concentration factor of 40. The limit of detection (LOD) of the method was 0.26 μg L^{-1}, while the relative standard deviation (RSD) for eleven replicated measurements was 3%. Accuracy of the method was assessed by analyzing SLEW-3 (estuarine water) and TM-23.3 (lake water) certified reference materials. In addition to the selectivity of the synthesised material for nickel(II) ions against other transition metal ions and major alkaline and alkaline-earth metals (Na^+, K^+, Mg^{2+} and Ca^{2+}) in seawater, it can be stated that the salt matrix is efficiently removed by using the proposed IIP-SPE procedure.

Keywords

Ionic imprinted polymer, precipitation polymerization, 5-vynil-8-hydroxyquinoline, nickel, trace elements, seawater, solid phase extraction, inductively coupled plasma - optical emission spectrometry.

1. Introduction

Solid phase extraction is one of the most used techniques for isolating trace elements from liquid samples before analytical determinations mainly by atomic spectrometric methods. However, the main disadvantage of conventional SPE sorbents for trace elements, such as C18, ion-exchange resins and size-exclusion phases, is the lack of selectivity, leading to co-extraction of matrix concomitants with the target analytes [1]. This fact is specially important when coping with seawater samples because of the high salt content which is an important source of spectral and matrix interferences [2-4]. However, specific SPE sorbents, as immunosorbents (ISs) and molecularly imprinted polymers (MIPs), can avoid this problem by providing selective extraction of such analytes [5].

Synthetic MIPs offer artificial recognition sites which are able to specifically rebind a target molecule in the presence of other similar compounds. These materials can be easily prepared by polymerization of functional and cross-linking monomers around a template molecule (target). As result, a highly cross-linked three-dimensional network polymer is obtained. After polymerization, the template molecule is leached, and the polymer offers binding sites with shape, size and functionalities complementary to target analyte [6]. Most of the MIPs are prepared by bulk polymerization. After this polymerization process, the material obtained has to be ground and sieved to obtain certain uniformity in particle size. These stages commonly lead to loses of fine particles of the synthesised material. In addition, the particles obtained are normally irregular in size and shape, and some binding sites are partially destroyed during grinding which leads to a considerable loss of loading capacity of the imprinted polymer [7]. To overcome these problems, different polymerization methods (suspension, emulsion, dispersion and precipitation) have been proposed for synthesizing MIPs [8]. These synthesis techniques have emerged as appealing methods for producing high-quality imprinted products [9-11], because crushing and sieving steps are avoided and higher yields of reaction are obtained.

IIPs are similar to MIPs but recognize metal ions after imprinting. For the last years different IIPs have been synthesised for recognizing lanthanides [12-17], actinides [18-22], noble metals [23,24] and transition elements such as copper [25-27], cadmium [28-30], selenium [31], nickel [32-34], aluminium [35,36], zinc [37], lead [38], iron [39,40], chromium [41] and mercury [42]. Different approaches for the synthesis of IIPs have been reviewed by Rao et al. [43], and four mechanisms can be established: (i) cross-linking of bifunctional reagents with linear chain polymers, (ii) chemical immobilization of vinylated ligands in the polymer matrix, (iii) surface imprinting by

emulsion polymerization, and (iv) trapping of a non-vinylated chelating agent inside the polymer matrix. The first three approaches use vinylated reagents which form complexes with the template (metal ion) and can polymerise through the vinyl groups. In such cases, the complexing ligand is chemically immobilized in the polymeric matrix [25,28,29,31,32,36,39]. However, the use of the trapping technique implies that the ligand (a non vinylated reagent) is not chemically bonded to the polymer chains, but it is still trapped inside the polymeric matrix. Different non-vinylated reagents have been successfully applied for synthesising IIPs by using the trapping approach [13,14,16,19-21,23,24,42]. However, low selectivity has been reported for synthesised IIPs based on 8-HQ prepared for recognition of Ni [44]. In this case, the IIP offered recognition capabilities for other transition metals, although the shape and size of such ions was different to those offered by the template [44]. This fact could be explained by keeping in mind that the complexing agent is trapped into the polymeric matrix, but is not linked to the polymer chains, so the imprinting cavities can show certain “flexibility”, offering specific interactions between their functional groups and other trace elements rather than the template.

The objective of the current work has been the synthesis of a new IIP for Ni recognition/preconcentration by using 5-VHQ instead of 8-HQ, to fix the complexing agent to the polymeric matrix, and so improving selectivity. This vinylated reagent was first used by Buono et al. [45,46] for obtaining poly-5-vynil-8-hydroxiquinoline (PVO) as a polymeric material for precipitating trace metals from saline waters [45]; and also for preparing chelating columns by coating on controlled pore diameter glass beads [46]. However, low selectivity was reported for these early applications [45,46]. In the current study, a selective polymeric material based on ionic imprinting methodology was prepared by co-polymerising 5-VHQ with DEM and DVB. The procedure leads to

a solid support which is able to selectively rebind nickel(II) in the presence of other trace elements in complex matrices such as seawater.

2. Experimental Section

2.1. Instrumentation

Determinations were performed by using an Optima 3300 DV inductively coupled plasma atomic emission spectrometer (Perkin Elmer, Norwalk, CT, USA) equipped with an auto sampler AS 91 (Perkin Elmer) and a Gem-Cone cross-flow nebulizer (Perkin Elmer). The polymerization process was carried out on a temperature-controllable incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA). IIPs were packed into 5mL cartridges (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA) and an 8-way Gilson peristaltic pump (Gilson, Villiers, France) equipped with 3.18 mm i.d. Tygon tubes (Gilson) was used to drive samples through the IIP cartridges. An ORION 720A plus pH–meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements. Scanning electron microscope LEO-435VP from LEICA Microsystems (Cambridge, UK), energy dispersive X-ray fluorescence spectrometer (laboratory-made by RIAIDT, University of Santiago de Compostela), EA 1108 element analyser (Fisons, Ipswich, UK) and EA 1108 element analyser (Carlo Erba, Milan, Italy) were used for IIP characterization.

2.2. Reagents

Ultra-pure water of resistivity 18MΩcm obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA) was used to prepare the solutions. Analytical grade $NiCl_2 \cdot 6H_2O$ and 69% nitric acid were obtained from Panreac (Barcelona, Spain). Ammonia 25% (v/v) and ammonium chloride were purchased from Merck (Darmstadt,

Germany). Single standard solutions (1000 mg L^{-1}) of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn were from Merck. 2-(diethylamino) ethyl methacrylate (DEM) was from Sigma-Aldrich (Steinheim, Switzerland). HPLC grade acetonitrile and toluene were purchased from Scharlab (Barcelona, Spain). 5-vynil-8-hydroxyquinoline (5-VHQ) was synthesised at the "Unidad de Química Combinatoria" at the University of Barcelona (Barcelona, Spain). RMN spectra of 5-VQH is given in Figure S1 (Supplementary data). Divinylbenzene-80 (DVB) from Sigma-Aldrich was treated in order to remove the polymerization inhibitor by passing a few millilitres of the reagent through a mini-column containing 0.5g of neutral alumina (Sigma-Aldrich). 2,2´-azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at -20°C after dissolving the reagent in methanol (Merck) at 50 – 60°C. After purification, this reagent was stored at 4°C. Lake water (TM-23.3) certified reference material was purchased from the National Water Research Institute of Canada. Estuarine seawater (SLEW–3) certified reference material was obtained from the National Research Council of Canada.

All glass and plastic material was rigorously cleaned and kept into 10% (v/v) nitric acid for 48h. The material was then rinsed three times with ultra-pure water before being used.

2.3. Seawater collection

Seawater samples (1L) were collected from the Ría de Muros-Noia estuary (north-western Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were filtered through 0.45µm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then stored at low temperature until used.

2.4. ICP-OES measurements

Determinations were performed by ICP–OES (axial configuration) using the operating conditions and emission wavelengths given in Table 1. Aqueous standard solutions were prepared in 2.0M nitric acid covering metal concentrations within 0 - 4 mg L^{-1} for the optimization process and 0 - 0.25 mg L^{-1} for nickel IIP-SPE analytical performance studies.

2.5. Synthesis of nickel ionic imprinted polymer particles

The synthesis of IIP particles was carried out by mixing 0.076g of $NiCl_2 \cdot 6H_2O$, 0.13mL of DEM and 0.054g of 5-VHQ in cleaned glass test tubes. Then, 25mL of porogen (3:1 acetonitrile:toluene) were added, and the solution was stirred for 5 min and then filtrated. Finally, 1.13mL of DVB (cross-linker) and 0.150g of AIBN (initiator) were added, the glass tubes were purged with N_2 and immediately sealed just before thermal induction of the precipitation polymerization by increasing the temperature from room temperature to 60ºC over 2h. The temperature was then maintained at 60ºC whereas a low stirring rate of 33 rpm was used. After 24 h, the polymer was vacuum filtered, washed with acetonitrile and oven-dried at 40ºC. The mass of polymer obtained after polymerization was 0.530g and the efficiency of the polymerization process, considering 1 g as the theoretical amount of synthesized polymer, was 53%. Non imprinted polymer particles (NIP) were also prepared in the same way than IIP, but without the template.

2.6. IIP cartridge packing and template removal procedure

Around 300mg of the IIP were packed into a 5mL cartridge between Teflon frits. Afterware, nickel(II) ions (template) were removed from the polymer particles by extensive washing with 50mL of 2.0M nitric acid. Similarly, NIP (300mg) was also packed and subjected to the same washing pre-treatment.

Table 1. ICP–OES operating conditions.

General	Radiofrequency power / W	1300
	Peristaltic pump speed / mL min^{-1}	1.5
	Stabilization delay / s	45
	Number of replicates	4
	Nebulizer type	Cross flow
	Axial view	
Gas flows / L min^{-1}		
	Plasma	15.0
	Auxiliary	0.5
	Nebulizer	0.8
Detection wavelengths / nm		
	Al	396.153
	As	228.812
	Cd	228.802
	Co	238.892
	Cr	267.716
	Cu	327.393
	Fe	238.204
	Mn	257.610
	Ni	231.604
	Pb	224.688
	Sn	235.485
	Ti	336.121
	V	290.880
	Zn	213.857

2.7. IIP solid phase extraction procedure

Aqueous standard solutions (50 μg L^{-1}) were prepared in 100mL of 0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 9.0±0.1. Similarly, 100mL of acidified seawater samples were treated with 5.0M ammonia solution (approx. 1mL), to readjust the pH to 9.0±0.1. The solutions were passed through cleaned and conditioned IIP cartridges at a 3.0 mL min^{-1} flow rate by using a peristaltic pump. The cartridges were then rinsed with 2.5mL of the 0.1M/0.1M NH_4Cl/NH_3 buffer solution at pH 9.0±0.1, and then, the retained ions were subsequently eluted with two 1.25mL aliquots of 2.0M nitric acid solution at 1.5 mL min^{-1}. A pre–concentration factor of 40 was achieved under these operating conditions. After elution, the IIPs were treated with 10mL of Milli-Q water and then conditioned by passing 10mL of the 0.1M/0.1M NH_4Cl/NH_3 buffer solution at the working pH (9.0).

3. Results and discussion

3.1. Characterization studies

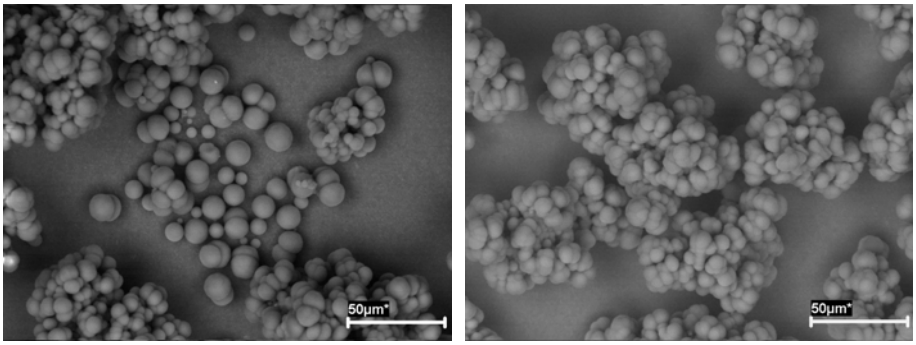
3.1.1. Scanning electron microscopy

Scanning electron microscopy (SEM) pictures were taken from IIP and NIP (Figure 1) in order to study the morphology and the size of the materials synthesised. It can be observed that IIP consisted of agglomerates whereas NIP consisted of agglomerates and also spherical monodisperse particles of around 10 μm of diameter. The slightly different polymer morphology can be attributed to the presence of the template in the polymerization process.

3.1.2. Energy dispersive X ray fluorescence studies

EDXRF patterns for IIP material before and after leaching, as well as for the corresponding NIP were obtained (Figure 2). It can be observed that nickel was only present in the unleached IIP particles, and it was totally removed after the template removal procedure.

Figure 1. Scanning electron microscopy pictures for NIP (A) and IIP (B).



A B

Figure 2. Energy dispersive X ray fluorescence spectra for the unleached IIP, leached IIP and NIP.

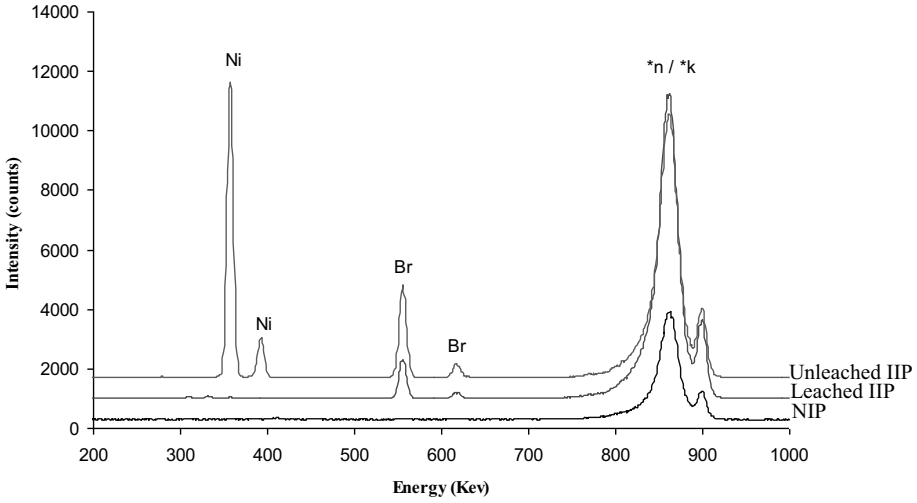


Table 2. Elemental composition of IIP and NIP.

	N		C		H		O	
	Calculated (%)	Found (%)	Calculated (%)	Found (%)	Calculated (%)	Found (%)	Calculated (%)	Found (%)
Unleached IIP	1.66	1.38	86.10	85.45	7.66	8.13	2.84	2.51
Leached IIP	1.69	1.77	87.63	86.76	7.79	8.08	2.89	2.48
NIP	1.69	1.40	87.63	88.59	7.79	8.38	2.89	1.72

3.1.3. Microanalysis studies

The elemental (H, C, N and O) composition of IIP and NIP are summarised in Table 2. There is a good agreement between calculated and experimentally found values of H, C, N and O confirming the incorporation of 5-VHQ to the polymeric network.

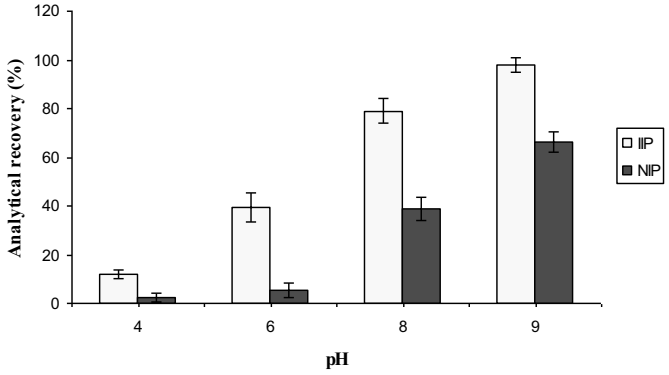
3.2. Optimization of nickel IIP-SPE from seawater

3.2.1. Effect of pH

A set of experiments was carried out (by triplicate) to determine the optimum pH for nickel SPE. The pre-concentration procedure (section 2.7) was applied to 100mL of an aqueous solution containing 50 μg L^{-1} of Ni(II) at four different pHs (from 4.0 to 9.0) fixed by using 0.1M/0.1M NH_4Cl/NH_3 buffer solutions. The same experiment was carried out by using the NIP.

Figure 3 shows the effect of the pH on the analytical recovery of Ni after IIP/NIP-SPE. It can be observed that Ni(II) is quantitatively retained in the IIP at pH 9.0, whereas the analytical recoveries for the non-imprinted polymer are quite lower for all the tested pHs than when using the IIP.

Figure 3. Effect of the pH on the nickel analytical recovery for IIP and NIP.



3.2.2. Effect of the load and elution flow rate

Different experiments were performed in order to find the optimum load and elution flow rates. 100mL aliquots of an aqueous solution containing 50 μg L^{-1} of Ni(II) were subsequently passed through IIP cartridges at 3.0, 6.0, 9.0 and 12.0 mL min^{-1}. Elution was fixed at 1.5 ml min^{-1} using a 2.0M nitric acid solution. Results have shown that Ni analytical recovery decreases for load flow rates larger than 3.0 mL min^{-1}.

Similarly, 100mL aliquots of an aqueous solution containing 50 μg L^{-1} of Ni(II) were passed at 3.0 mL min^{-1}, and eluted with a 2.0M nitric acid solution at 1.5, 3.0, 4.5 and 6.0 mL min^{-1}. Results have shown that Ni analytical recoveries decrease for elution flow rates larger than 1.5 mL min^{-1}.

In order to achieve the highest nickel analytical recovery, a 3.0 mL min^{-1} load flow rate, and a 1.5 mL min^{-1} elution flow rate were chosen and used for further experiments.

3.2.3. Effect of the nitric acid volume and concentration for elution

Different experiments were performed (by triplicate) in order to find the optimum nitric acid concentration and volume for the eluting solution. After sample loading and rinsing

(section 2.7), the retained analytes were eluted by passing volumes of 2.5mL of nitric acid at different concentrations (from 2.0M to 5.0M). After measuring by ICP-OES against an aqueous calibration prepared at the same nitric acid concentration, similar analytical recoveries were obtained for all cases; thus, an eluting solution of 2.0M nitric acid was selected for further studies.

Finally, volumes of 2.5, 5.0, 7.5 and 10mL of a 2.0M nitric acid solution were tested and quantitative nickel analytical recoveries were reached under all nitric acid volumes. In order to achieve the highest pre-concentration factor, an eluting volume of 2.5mL (pre-concentration factor of 40) was chosen. It should be mentioned that the elution process must be done in two steps to achieve the highest analytical recovery. Therefore, two 2.0M nitric acid aliquots of 1.25mL each one were subsequently passed through the IIP cartridges.

3.2.4. Retention capacity

300 mg of polymer were saturated with nickel(II) ions under optimum conditions to determine the retention capacity of the polymer (maximum amount of nickel(II) ions retained per gram of IIP). Several 3.0mL aliquots of a 200 μg mL^{-1} Ni(II) solution were passed subsequently through the polymer and the nickel content in the eluates was measured by ICP-OES. The retention capacity of the polymer was calculated to be 1.98 mg g^{-1}. The theoretical retention capacity is 18.66 mg g^{-1}; therefore, the calculated retention capacity is around 11% of the theoretical retention capacity. This result agrees with reported retention capacities for MIPs, approximately 10% of the theoretical capacity [47].

3.2.5. Breakthrough volume

Different volumes (from 100 to 500mL) of an aqueous solution containing 50 μg L^{-1} of nickel(II) were passed through the cartridges (300 mg) at optimum parameters (section 2.7). All experiments were performed by triplicate and the nickel analytical recoveries found are listed in Table 3.

Table 3. Breakthrough volume.

Sample volume (mL)	Ni analytical Recovery (%)[a, b]
100	100 ± 3
200	104 ± 3
300	100 ± 4
400	91 ± 3
500	83 ± 5

(a) n = 3.

(b) IIP mass, 300 mg

It can be observed that sample volumes up to 300mL can be passed through cartridges packed with 300mg of the polymer without appreciable losses of analytical recovery. This offers a pre-concentration factor of 120 when eluting with 2.5mL of the eluting solution. However, in order to achieve a reasonable pre-concentration factor in a short time, a sample volume of 100mL was chosen, so one sample can be pre-concentrated in approximately 35 minutes with a pre-concentration factor of 40. Therefore, eight samples can be simultaneously pre-concentrated in 35 minutes by using 8 polymer cartridges and an 8-way peristaltic pump.

3.2.6. Cross-reactivity among nickel and other trace elements

To evaluate the cross-reactivity among nickel and other selected trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Pb, Sn, Ti, V, and Zn) 100mL of an aqueous solution containing 50 μg L^{-1} of nickel(II) and the other trace metals were passed through the IIP and the NIP (by triplicate) after fixing the pH at 9.0. Results, expressed as percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$), are listed in Table 4.

Table 4. Percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$) for IIP and NIP.

	IIP			NIP		
Element	E (%)[a]	D[b]	$S_{Ni/M}$[c]	E (%)[a]	D[b]	$S_{Ni/M}$[c]
Ni	98.5	65.7	-	73.2	2.73	-
Cu	74.9	3.0	22.01	76.0	3.17	0.86
Pb	76.2	3.2	20.51	75.0	3.00	0.91
Zn	70.8	2.4	27.08	74.4	2.91	0.94
As	80.6	4.2	15.81	48.9	0.96	2.85
Al	11.6	0.1	500.43	11.1	0.12	21.88
Cd	80.1	4.0	16.31	48.6	0.95	2.89
Cr	79.5	3.9	16.93	83.9	5.21	0.52
Fe	84.8	5.6	11.77	85.0	5.67	0.48
Mn	69.6	2.3	28.68	15.1	0.18	15.36
V	65.4	1.9	34.74	53.2	1.14	2.40
Sn	82.3	4.6	14.12	85.0	5.67	0.48
Co	62.6	1.7	39.23	59.5	1.47	1.86
Ti	79.3	3.8	17.14	83.1	4.92	0.56

(a) $E\ (\%) = (A_2/A_T)\times 100$.

(b) $D = (A_2/A_1)$.

(c) $S_{Ni/M} = D_{Ni}/D_M$.

A_1 =Amount of metal ion in aqueous solution at equilibrium; A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium. A_T = Total amount of metal ion used in extraction. D_{Ni} = Distribution ratio for Ni; D_M = Distribution ratio for M (M = Cu, Pb, Zn, As, Al, Cd, Cr, Fe, Mn, V, Sn, Co and Ti).

From these results, the following observations can be made:

1) The IIP material has shown good selectivity for nickel over other transition metals, including Cu, Pb and Zn. As demonstrated in previous works [44], Ni-based IIP synthesized by the same precipitation polymerization technique but using a non-

vinylated chelating agent (8-HQ) instead of a vynilated chelating ligand (5-VHQ), offered imprinting properties for the template (nickel(II)) and also for copper(II), lead(II) and zinc(II) with quantitative analytical recoveries. By using 5-VHQ as a bi-functionalised ligand, active groups of the hydroxyquinoline ring are linked to the polymer chains forming rigid imprinting cavities. In this manner, the "flexibility" of the imprinting cavities offered by IIPs based on 8-HQ [44] is avoided, and selectivity for the target ion is highly enhanced.

2) The NIP particles have shown no selectivity for nickel(II) ions over other selected transition metals.

3) The highest selectivity was obtained for the Ni/Al pair, which indicates the two elements having the most important differences in terms of coordination with 5-VHQ and DEM.

4) Although IIP has not shown imprinting properties for Cu, Pb, Zn, Cr, Fe, Sn, Co and Ti, there are some unspecific interactions between these transition metals and the polymeric material with analytical recoveries close to 75%.

3.2.7. Effect of major components from seawater

In order to study the interactions between the polymeric material and the major metals present in seawater (Na^+, K^+, Ca^{2+} and Mg^{2+}), 100mL of a seawater sample was subjected eleven times to the proposed procedure (section 2.7) and the concentrations of these elements were determined in the eluates by ICP-OES. After pre-concentration, values of around 24, 2, 22 and 6 mg L^{-1} were found for Na, K, Mg and Ca, respectively. Since the concentrations of Na, K, Mg and Ca in seawater are around 11490, 399, 1293 and 413 mg L^{-1}, respectively [48], it can be concluded that there is not interaction between the polymer particles and the major elements present in seawater.

3.3. Analytical performance for nickel determination in seawater by IIP-SPE- ICP-OES

3.3.1. Calibration. Evaluation of matrix effect

A comparison between calibration in 2.0M nitric acid and a standard addition graph was established in order to study possible matrix effects. The standard addition graph was obtained after spiking a pool of eluates (obtained after the pre-concentration of four aliquots of the same seawater sample) with different nickel concentrations (0, 40, 80 and 160 μg L^{-1}). The mean and the standard deviation for the slopes of three standard addition graphs (7.16 ± 0.09 μg L^{-1}) and three external 2.0M nitric acid calibrations (7.41 ± 0.18 μg L^{-1}) were statistically compared by using the Cochran'C and Bartlett's tests at a 95.0% (comparison of variances), and the ANOVA test (comparison of means). Slopes for external 2.0M nitric acid calibration and standard addition graphs were statistically similar, so the salt matrix was efficiently removed during the pre-concentration stage. This result agrees with the fact that there is no interaction between major ions present in seawater and the polymeric material (Section 3.6). Therefore, a simple calibration with nickel standard solution in 2.0M nitric acid is enough to perform seawater analysis for nickel, avoiding the performance of a standard addition graph.

3.3.2. Sensitivity of the method

The limits of detection and quantification were calculated according to LOD = (3*SD)/m and LOQ = (10*SD)/m, where SD is the standard deviation of eleven measurements of a procedural blank (acidified Milli-Q water treated as a sample) and m is the slope of an external 2.0M nitric acid calibration graph. The limit of detection was calculated to be 0.26 μg L^{-1} for a pre-concentration factor of 40. Similarly, the limit of quantification was calculated to be 0.87 μg L^{-1}. Such LODs and LOQs are low enough to determine nickel in unpolluted seawater samples. These values are similar to those reported by other authors when using IIPs combined with ETAAS (0.3 μg L^{-1} [32] or 0.18 μg L^{-1} [34]) or ICP-OES (0.16 μg L^{-1} for [33]) detection.

3.3.3. Repeatability and accuracy of the method

To assess the repeatability of the overall procedure (IIP-SPE and ICP-OES determination) a seawater sample was analysed eleven times by the proposed procedure (section 2.7), giving a mean nickel concentration of 0.93 μg L^{-1} and a R.S.D of 3%, which confirms good repeatability of the overall procedure.

The accuracy of the method was verified by studying the analytical recovery and by analyzing two certified reference materials with different salinities (SLEW-3 and TM-23.3). Analytical recovery was assessed for three nickel concentration levels, after spiking three different aliquots from the same seawater sample with 1.0, 2.0 and 4.0 μg L^{-1} of Ni. Each nickel concentration was performed by triplicate. The analytical recoveries obtained were 99 ± 3 %, 98 ± 3 % and 99 ± 3 % for 1.0, 2.0 and 4.0 μg L^{-1} of Ni, respectively, so it can be concluded that complete analytical recovery was obtained for all the nickel concentration levels studied. SLEW-3 (estuarine water) and TM-23.3 (lake water) certified reference materials were analyzed by duplicate by using a sample volume of 100mL (pre-concentration factor of 40). Results, listed in Table 5, reveal good agreement between found concentrations and certified values for both certified reference materials. This fact has been verified after applying the t-test for means comparison.

Table 5. Analysis of certified reference materials (n=2)

SLEW-3		TM-23.3	
Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})
1.23 ± 0.07	1.17 ± 0.31	5.4 ± 1.1	6.1 ± 0.4

3.3.4. Study of the lifetime of the Ni-IIP support

Different Ni-IIP cartridges were tested in order to know the number of sequential loading/elution cycles that can be performed without losses on pre-concentration efficiency. These experiments were carried out by treating aqueous standard solutions containing 1.0 μg L^{-1} of Ni (II), and calculating the analytical recovery after ICP-OES measurement. Before passing each Ni(II) aqueous standard solution, the same cartridges containing the IIP, were previously used five times for pre-concentrating different estuarine water samples. It has been found that quantitative nickel recoveries (higher than 95%) were reached within the fifty loading/elution cycles, and analytical recoveries lower than 95% were obtained after fifty SPE cycles. Therefore, IIP cartridges can be used at least fifty times without losing of efficiency of the adsorbent IIP for nickel determination.

4. Conclusions

The synthesised Ni based IIP by using a vynilated bi-functionalised reagent (5-VHQ) offers higher selectivity for the target ion (nickel(II)) than that offered by Ni-based IIPs synthesised in the presence of 8-HQ [44]. This result is attributed to the fact that the vynilated ligand is linked to the polymer chains rather than being trapped into the polymeric matrix. Therefore, rigid imprinting cavities are obtained and high nickel(II) ions recognition capabilities are achieved. These results suggest that a low-selective complexing agent without vynil groups, such as 8-HQ, can be transformed into a selective agent by adding a vynil group and subjecting it to an ionic-imprinting polymerization process. In addition to selectivity over other transition metals, the new IIP material has not offered affinity for major elements in seawater, such as sodium and potassium, allowing the determination of Ni in seawater by using external aqueous calibrations.

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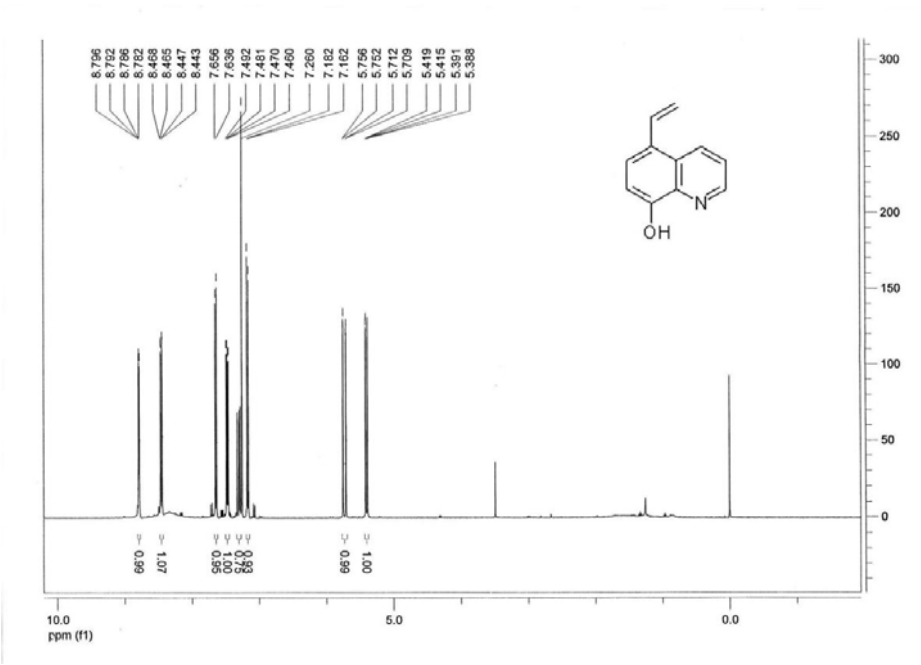
6. Supplementary data

Figure S1. RMN spectra of 5-vynil-8-hydroxyquinoline (5-VQH).



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Cu, Ni, Pb, Mn, Sn, Fe, Al and Zn distribution in the Ría de Arousa. Study before and after the Prestige oil-tanker sinking

Jacobo Otero-Romaní, Diana Bello-Currás, Antonio Moreda-Piñeiro,

Pilar Bermejo-Barrera

RESUMEN

El 19 de noviembre de 2002, el petrolero "Prestige" se hundió a 133 km del Cabo Finisterre, llevando en su interior 77.000 toneladas de fuel-oil N º 6. El vertido afectó a las costas de Galicia, la costa norte de España, la costa norte de Portugal y el suroeste de Francia. El combustible Nº 6 (Bunker C) es un tipo de fuel-oil residual que se utiliza para diversos propósitos industriales, incluida la producción de energía eléctrica y como combustible de propulsión de embarcaciones marinas. La USEPA (United States Environmental Protection Agency) ha informado de que la concentración media de metales pesados en este combustible residual es de 3,8 mg l^{-1} de Al, 18 mg l^{-1} de Fe, 3,5 mg l^{-1} de Pb y 1,3 mg l^{-1} de Zn. Otros autores han indicado que también contiene niveles de cobre de hasta 0,88 mg l^{-1} y otros metales pesados como Mn (2 mg l^{-1}), Sn (3 mg l^{-1}) y níquel (de 3,0 a 118 mg l^{-1}).

Durante el presente trabajo de investigación se ha determinado la concentración de dichos elementos traza en las aguas de la Ría de Arousa y se ha evaluado si el accidente del Prestige ha tenido alguna influencia sobre los niveles de concentración de dichos metales en la Ría. Las muestras fueron recolectadas durante dos campañas de muestreo realizadas el 18 de marzo de 2002 y el 7 de febrero de 2003, tan solo 8 meses antes y 3 meses después del accidente del petrolero.

Los elementos traza han sido determinados simultáneamente mediante ICP-OES, tras la complejación de los metales en disolución con 8-hidroxiquinolina (8-HQ) a pH alcalino y la preconcentración de las muestras de agua de mar en cartuchos comerciales de sílice C18.

Los valores obtenidos resultaron ser similares a los publicados por otros autores para las Rías gallegas, lo que indica que el vertido del Prestige no ha tenido una gran influencia sobre la concentración de dichos elementos en las aguas de la Ría de Arousa, donde no se observó contaminación por metales pesados.

De los resultados obtenidos podemos indicar que, en general, las concentraciones más elevadas de elementos traza se encontraron en las aguas del río Ulla y las estaciones ubicadas en su desembocadura. En las estaciones correspondientes al estuario los niveles de Cu, Ni, Pb y Mn resultaron ser ligeramente más elevados en las aguas superficiales en 2003, mientras que en las muestras de profundidad las concentraciones de Cu y Pb resultaron ser ligeramente superiores en el año 2002 para la mayoría de las estaciones estudiadas.

El trabajo incluye mapas de distribución de cada elemento traza, Análisis de Componentes Principales y Analisis de Cluster para las muestras de agua superficiales.

Este trabajo de investigación ha sido enviado para su publicación en la revista **International Journal of Environmental Analytical Chemistry** en octubre de 2009.

Cu, Ni, Pb, Mn, Sn, Fe, Al and Zn distribution in the Ría de Arousa. Study before and after the Prestige oil-tanker sinking

Jacobo Otero-Romaní, Diana Bello-Currás, Antonio Moreda-Piñeiro, Pilar Bermejo-Barrera

Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. University of Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain.

Abstract

The behaviour of several trace elements (Cu, Ni, Pb, Mn, Sn, Fe, Al and Zn) at the Ría de Arousa (Galicia, NW of Spain) and the influence of the Prestige accident at the estuary were evaluated. The Ría de Arousa is one of the most productive estuaries in the world, with near 69% of the Galician mussel rafts. Samples were collected during two sampling campaigns, the 18th of March 2002 and the 7th of February 2003, just 8 months before and 3 month after the oil-tanker accident. Trace elements were simultaneously determined in seawater samples by ICP-OES after complexation with 8-Hydroxyquinoline (8-HQ) at alkaline pH and preconcentration onto commercial C18 cartridges. The highest trace elements concentrations were found at the Ulla River and the stations located at its mouth. The values obtained at the estuary were similar to those previously reported by other authors for the Galician Rías, indicating that the Prestige oil spillage had no serious influence on the studied trace metal levels at the Ría de Arousa waters, where no heavy-metal pollution was observed. Trace element distribution maps, principal components analysis and cluster analysis were also performed for shallow waters at years 2002 and 2003.

Keywords

Seawater, Ría de Arousa, Galicia, Prestige, Trace metals, Solid-Phase Extraction, C18, 8-Hydroxiquinoline, ICP-OES

1. Introduction

Sea-waters have remained unpolluted for millenniums, but this situation has been changing gradually over the past century due to human activity. During these years, various urban and industrial settlements have been produced on coasts and rivers, which results in a progressive increasing in water pollution. Galicia (North-West of Spain) is a community particularly sensitive to this reality, having a coastline with numerous estuaries, where waters are particularly stalled, being as a result, more sensitive to pollutants discharged. Moreover, the Galician coast support a large volume of maritime traffic, which constitutes an important risk factor, as has been found after the sinking of large ships that carried the most diverse materials (oil, chemicals, etc ...).

The Galician Rías is one of the richest shellfish and fishing areas in the world, due to its rich waters and to the existence of fish and shellfish hatchery. It is well known that shellfish filter large amounts of water and is, therefore, particularly sensitive to the concentration of pollutants in their bodies such as heavy metals. If waters are polluted, there is a big risk that these metals absorbed by shellfish, pass to the food chain, with the consequent risk to public health that entails.

In 2002, Spain produced the 17.3% of the mussel cultivated in the world (47% of the European mussel harvest) [1]. Around 95% of the Spanish mussel harvest is carried out in Galicia and Arousa, in particular, is the most productive Galician Ría with 69% of the Galician mussel rafts, covering a surface area of 27 km^2, which represents 11% of the total surface area of the ría [2]. The Ría de Arousa is a large bay, where it can be

distinguished between the lower and the upper estuary. The upper estuary includes a channel were fluvial and marine water mix, and the influence of river water is only important in its mouth [3].

The 19th November of 2002, the oil tanker "Prestige" sank at 133 Km from Cape Finisterre, carrying 77,000 tons of heavy fuel oil No.6. The spill affected the Galician coast, the northern coast of Spain, the northern Portuguese coast, and the southwest of France. The oil didn't enter the Rías Baixas but there was heavy oil slick pollution at the entrance of the estuaries, especially at the area of the Spanish National Park of the Atlantic Islands (Sálvora, Ons and Cíes) and the area of Corrubedo. Due to the accident, the Galician government ordered a ban on shellfish harvesting in the Rías Baixas, that was lifted on the 30th of January of 2003 [4].

Fuel No.6 (or Bunker C) is the last residue in refinery operation that is used for various industrial purposes, including the production of electrical power and for propulsion as a marine vessel fuel. This kind of fuel is highly viscous, its biodegradability is low and emulsions formed in water are very stable. After 2 months in the sea, losses by solution or evaporation are less than 5 % [5]. USEPA (1993) reported an average concentration in residual fuel of 3.8 mg L^{-1} for Al (values ranging from 0.3 to 19 mg L^{-1}), 18 mg L^{-1} for Fe (values ranging from 2 to 2200 mg L^{-1}), 3.5 mg L^{-1} for Pb (values ranging from 0.1 to 8.0 mg L^{-1}) and 1.3 mg L^{-1} for Zn (values ranging from 0.6 to 35 mg L^{-1}) [6]. Lloyd's Register (1995) reported values ranging from 0.12 to 0.88 mg L^{-1} for copper concentration in residual fuel, whereas Raymond *et al.* reported an average concentration of 2 mg L^{-1} for Mn and 3 mg L^{-1} for Sn [6]. Graziano and Daniels reported values ranging from 3.0 to 118 mg L^{-1} for nickel concentration in residual fuel [6]. Trace metal contents in the Galician estuaries have been previously reported by Prego and Covelo-García [7] and Beiras *et al.* [8]. The impact of the Prestige oil spill on

trace element levels in seawater have been also studied by J. Santos-Echeandía *et al.* for Cu, Ni and V [9], Prego and Cobelo-García for Cd, Cu, Pb [10] and Zn [11], and E. Peña-Vazquez *et al.* for As and Sb [12].

The main objective of this paper is the characterization of copper, nickel, lead, manganese, tin, iron, aluminium and zinc at the Ría de Arousa to evaluate if their concentration levels have increased after the Prestige sank.

2. Experimental

2.1. Instrumentation

All measurements were performed by using an Optima 3300 DV inductively coupled plasma optical emission spectrometer (Perkin-Elmer, Norwalk, USA) equipped with a GemCone cross flow nebulizer (Perkin-Elmer) and a cyclonic spray chamber (Perkin-Elmer). Samples were driven through the C18 cartridges (Sep-Pack Plus – 360 mg, Waters, Milford, MA, USA) by employing an 8-way Gilson peristaltic pump (Gilson, Villiers, France) equipped with 3.18mm i.d. Tygon tubes (Gilson). All experiments were performed in a Class-100 clean fume hood (Telstar S.A., Terrassa, Spain) in order to avoid contamination of samples and reagents.

2.2. Reagents

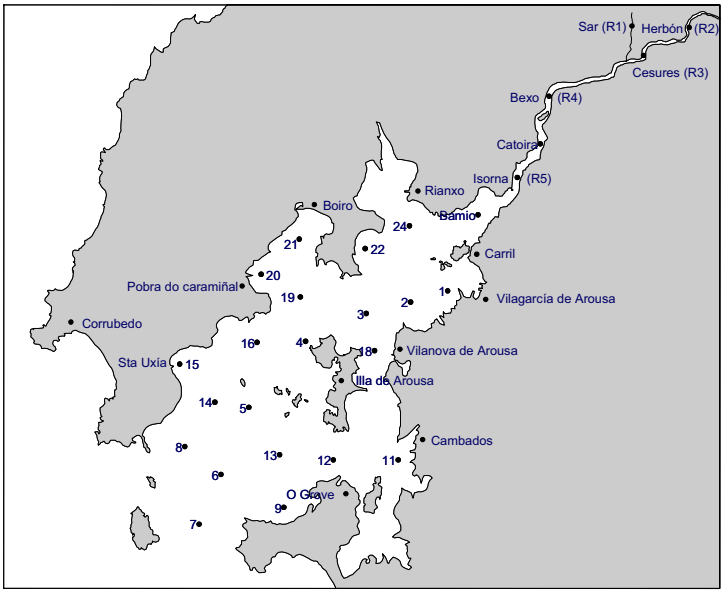
Ultra-pure water of resistivity 18 MΩcm obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA) was used to prepare all the solutions. High purity nitric acid and hydrochloric acid were from Panreac (Barcelona, Spain). High purity ammonia and analytical grade methanol were purchased from Merck (Darmstad, Germany). 8-Hydroxyquinoline solution (0.5 M) was prepared from analytical grade reagent (Merck) by dissolving in a 2.0M hydrochloric acid solution. Ammonium acetate solution (0.01 M) was prepared from analytical grade ammonium acetate (Merck) by

dissolving in Milli-Q water. Single standard solutions (1000 mg L^{-1}) of Al, Cu, Fe, Mn, Ni, Pb, Sn and Zn were purchased from Merck. Estuarine seawater (SLEW-3) and coastal seawater (CASS-3) certified reference materials were obtained from the National Research Council of Canada. Lake water (TM-24) certified reference material was purchased by the National Water Research Institute of Canada. All glass and plastic material were cleaned and kept into 10% (m/m) nitric acid for at least 48 h. The material was then rinsed three times with Milli-Q water.

2.3. Seawater collection

Samples were collected during two sampling campaigns, in 2002 (18th of March) and in 2003 (7th of February), on board R/V Mytillus (or on a rubber dinghy upstream on the river) and using a Niskin sampler. Sampling points are located in Figure 1. Samples were introduced in 100 mL polyethylene bottles previously kept in 10% nitric acid for 48 hours and rinsed with milli-Q water.

Figure1. Sampling stations at the Ría de Arousa and Ulla River.



At each sampling point, three replicates were gathered from the surface water and at different depths. Samples were taken from surface, mid-depth and close to the estuary bed in the axis of the Ría de Arousa. After collection, seawater samples were filtered with 0.45 μm polycarbonate membrane Nucleopore filters (Millipore), and then acidified at pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Samples were stored at –20ºC until preconcentrations were performed.

2.4. Trace elements determination procedure

Al, Cu, Ni, Fe, Mn, Pb, Sn, and Zn were simultaneously determined by ICP-OES after complexation with 8-hydroxyquinoline at alkaline pH, and pre-concentration onto commercial C18 cartridges. The method had been optimized in a previous work [13] where accuracy was checked by performing different analytical recovery studies and analyzing several certified reference materials (CASS-3, SLEW-3 and TM-24). Detection and Quantification Limits, for a pre-concentration factor of 40, are listed in Table 1. It can be seen that these limits are low enough to determine trace metal levels in most unpolluted seawater samples.

Table 1. LOD and LOQ of the method.

Element	LOD / μg L^{-1}	LOQ / μg L^{-1}
Al	1.28	4.27
Cu	0.02	0.06
Fe	0.19	1.09
Mn	0.01	0.06
Ni	0.03	0.092
Pb	0.03	0.082
Sn	0.07	0.726
Zn	1.13	3.76

Although solid phase extraction implies metals separation from the seawater matrix, a certain matrix effect was observed when determining all elements, being necessary the establishment of a standard addition graph to perform seawater analysis. Each set of samples was measured together with 3 reagent blanks (acidified Milli-Q water treated as a sample) and the eluates obtained were frozen until element concentration was measured. The whole procedure is described in the following paragraphs.

2.4.1. C18 cartridge preparation

C18 cartridges were washed by passing 25 ml of 2.0M nitric acid solution at 1.5 ml min^{-1} flow rate, in order to remove trace metals adsorbed in the packing material. Then, the C18 cartridges were rinsed by passing 25 ml of Milli-Q water and they were activated by washing with 10 ml of methanol and rinsed with 10 ml of Milli-Q water. Finally, the activated silica was conditioned by passing 10 ml of 0.01M ammonium acetate (pH 7.0).

2.4.2. C18 cartridge solid phase extraction procedure

0.1 ml of 0.5M 8-HQ solution was added to 100 ml of acidified seawater samples. Then, a small volume (1–2 ml) of a 5.0M ammonia solution was added in order to readjust the pH to 8.0±0.1. Seawater samples were then passed through cleaned and conditioned C18 cartridges by using a peristaltic pump at a fixed flow rate of 6.0 ml min^{-1}. In order to remove the saline matrix, the cartridges were then rinsed with 10 ml of 0.01M ammonium acetate, and the adsorbed metal-complexes were eluted with 2.5 ml of 2.0M nitric acid, at a flow rate of 4.5 ml min^{-1}. Under these operating conditions, a pre-concentration factor of 40 was achieved.

2.4.3. ICP-OES measurements

Eluted trace elements were measured by ICP-OES by using the operating conditions and emission wavelength lines given in Table 2.

Table 2. Operating ICP–OES conditions

General	Radiofrequency power / W	1300
	Sample uptake rate / mL min^{-1}	1.5
	Stabilization delay / s	45
	Number of replicates	4
	Integration time / s	5
	Nebulizer type	Cross flow
	Axial view	
Gas flows / L min^{-1}		
	Plasma	15.0
	Auxiliary	0.5
	Nebulizer	0.8
Detection wavelengths / nm		
	Al	396.160
	Cu	327.393
	Fe	238.205
	Mn	257.610
	Ni	231.604
	Pb	224.688
	Sn	235.486
	Zn	213.858

The standard addition method was used, covering analyte concentrations from 0 to 0.4 mg L^{-1} for Cu, Ni and Pb; from 0 to 2.0 mg L^{-1} for Mn, Sn and Zn; and from 0 to 10.0 mg L^{-1} for Al and Fe, taking into account a pre-concentration factor of 40.

2.5. Treatment of data

Microsoft® Excel 2000 (Microsoft Ibérica S.R.L., Madrid, Spain) was used for the statistical treatment of data. Isoplot maps have been created using Surfer vs. 7.04 package (Golden Software. Inc, Colorado, EEUU). Principal components analysis was performed by using the Unscrumbler 7.01 statistical programme (CAMO ASA, Trondheim, Norway), and cluster analysis was performed by employing Statgraphics 5.0 (Manugistics, Inc., USA), using the Ward method as the method of formation of aggregates and the square of the Euclidean distance as the measure of similarity. To perform this analysis it is necessary that all variables have equal weight in the system,

so the concentration results were normalized using the "half-range and central value" transformation [14], which converts the minimum values to -1, the maximum values to +1, and the central values to 0, so we have values ranging from -1 to +1.

3. Results and discussion

Sample concentrations (in μg L^{-1}) are shown in Tables S1 (Cu) to S8 (Zn), which include values for all studied depths (Supplementary data). The results are expressed as the average ± the standard deviation, being n=3 for each sampling point. Corresponding element distribution maps are shown in Figures S1 (Cu) to S8 (Zn). These figures include concentrations found in the shallow waters of the Ría de Arousa and the Ulla River in years 2002 and 2003 (Supplementary data). From these tables and distribution maps, the following observations can be made:

3.1. Copper

1 - In 2002, the Cu concentration was between 0.17 and 2.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) with Cu levels ranging between 3 and 6.5 μg L^{-1}. The Cu concentration in depth samples was slightly higher at all the stations, with values ranging from 2 to 9 μg L^{-1}.

2 - In 2003, the Cu concentration was between 1 and 6.5 μg L^{-1} at all surface samples, except for stations 1 and 21 (located in front of "Vilagarcia de Arousa" and "Boiro", respectively), which had higher concentrations, ranging between 7.5 and 9 μg L^{-1}. In general, Cu levels in depth samples were similar to surface samples, with concentrations ranging between 1 and 4.5 μg L^{-1}.

3 – Generally, Cu concentration was slightly higher in 2003, in most of the surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) where the trend was reversed, and the Cu concentration was higher in 2002. In depth samples, the Cu concentration was slightly higher in most of the stations in 2002.

4 - All values obtained in our study were in the same range than those previously reported by Prego and Cobelo-García [7] for typical Cu concentrations at the Galician Rías (from 0.19 to 8.96 μg L^{-1}).

3.2. Nickel

1 - In 2002, the Ni concentration was between 0.18 and 1.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna), with Ni levels between 1.5 and 4.5 μg L^{-1}. The Ni concentration in depth samples was similar to surface samples, with concentrations ranging between 0.2 and 2.5 μg L^{-1}.

2 - In 2003, the Ni concentration was between 0.3 and 2.5 μg L^{-1} at all surface samples, except for stations 12 and 16 (located in front of "O Grove" and "Laxe", respectively), which had higher concentrations, ranging between 3 and 4.5 μg L^{-1}. In general, Ni levels in depth samples were similar to surface samples, with concentrations ranging between 0.25 and 1.5 μg L^{-1}.

3 – Generally, Ni concentration was slightly higher in most of the surface samples in 2003, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna), where the trend was reversed and the Ni concentration was slightly higher in 2002. In depth samples, the Ni concentration was very similar in 2002 and 2003, at most of the stations studied.

4 - All values obtained in our study were in the same range than those previously reported by Prego and Cobelo-García [7] for typical Ni concentrations at the Galician Rías (from 0.09 to 5.16 μg L^{-1}).

3.3. Lead

1 - In 2002, the Pb concentration was between 0.20 and 2.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna), with Pb levels ranging from 3 to 7 μg L^{-1}. The Pb concentration in depth samples was slightly higher at all stations, with values ranging from 2 to 9 μg L^{-1}.

2 - In 2003, the Pb concentration was between 0.5 and 6 μg L^{-1} at all surface samples, except for stations 1 and 21 (located in front of "Vilagarcia de Arousa" and "Boiro", respectively), which had higher concentrations, ranging between 7.5 and 8.5 μg L^{-1}. In general, Pb levels in depth samples were similar to surface samples, with concentrations ranging between 1 and 5 μg L^{-1}.

3 – Generally, Pb concentration was slightly higher in most of the surface waters in 2003, except for the river stations (Sar, Herbón, Cesuras, Bexo and Isorna) where Pb concentration was slightly higher in 2002. In depth samples, Pb concentration was slightly higher in most of the stations in 2002.

4 - Some values obtained in our study were higher than those previously reported by Prego and Cobelo-García [7] for typical Pb concentrations at the Galician Rías (from 0.23 to 2.9 μg L^{-1}). However, Beiras *et al.* have reported larger concentrations (until 39 μg L^{-1}) at Ría de Pontevedra [8].

3.4. Manganese

1 - In 2002, the Mn concentration was between 0.67 and 3.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the mouth of the Ulla River), which exhibited higher concentrations. The Mn levels in these samples ranged between 6 and 9 μg L^{-1} for the stations located at the mouth of the river, and 31 to 38 μg L^{-1} for the river samples. In general, the Mn concentration in depth samples was similar to the surface samples, with concentrations ranging between 0.5 and 7 μg L^{-1}.

2 - In 2003, the Mn concentration was between 1.5 and 9.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River) with higher concentrations of Mn, between 11 and 24.5 μg L^{-1}. In general, Mn levels in depth samples were lower than surface samples, with values ranging between 0.35 and 5 μg L^{-1}.

3 - Generally, Mn concentration was slightly higher in most of the surface samples in the year 2003, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) where the trend was reversed and the Mn concentration was higher in 2002. In depth samples, the Mn concentration was very similar in 2002 and 2003, at most of the stations studied.

4 – No Mn levels at the Galician Rías were found in the literature, so it was not possible to establish a comparison with previously reported Mn concentrations.

3.5. Tin

1 - In 2002, the Sn concentration was between 2 and 10 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the mouth of the Ulla River), which exhibited higher concentrations. Sn levels at these stations ranged from 14 to 21 μg L^{-1} for samples located at the mouth of the river, and 25 and 43 μg L^{-1} for the river samples. Sn concentration in depth samples was similar to the surface samples, with concentrations ranging between 1 and 8.5 μg L^{-1}, except for 1, 22 and 24 stations (located at the mouth of the Ulla River) which exhibit values between 12.5 and 21 μg L^{-1}.

2 - In 2003, the Sn concentration was between 1 and 10 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River) which exhibited higher concentrations. Sn levels at these stations ranged between 10.5 and 27.5 μg L^{-1}. In general, Sn levels in depth samples were similar to surface samples, with concentrations ranging from 1.5 to 11 μg L^{-1} at all stations, except for stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla river) with concentrations between 11.5 and 14.5 μg L^{-1}.

3 - Generally, the Sn concentrations were similar in the two sampling campaigns (2002 and 2003), both in surface samples and depth samples, showing the most significant

differences in surface river samples (Sar, Herbón, Cesures, Bexo and Isorna), where Sn concentrations were markedly higher in 2002.

4 – All values obtained in our study were in the same range than those previously reported by Bermejo-Barrera *et al.* [15] for Sn concentrations at the Ría de Arousa (from 1.8 to 35.2 μg L^{-1}).

3.6. Iron

1 - In 2002, the Fe concentration was between 11 and 45 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the mouth of the Ulla River), which exhibited higher concentrations. The Fe levels at these stations ranged from 71 to 79 μg L^{-1} for samples located at the mouth of the river, and from 131 to 224 μg L^{-1} for the river samples. The Fe concentration in depth samples was similar to surface samples, with values ranging between 9.5 and 41.5 μg L^{-1} for all stations, except for stations and 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River) with values between 65 and 70 μg L^{-1}.

2 - In 2003, the Fe concentration was between 9.5 and 49.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River) with higher Fe concentrations. The levels of Fe in these stations ranged between 52 and 150.5 μg L^{-1}. In general, Fe levels in depth samples were similar to surface samples, with values ranging between 13.5 and 47 μg L^{-1} at all stations, except for stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River), with values between 55 and 66.5 μg L^{-1}.

3 - Generally, Fe concentrations were similar in the two sampling campaigns (2002 and 2003), both in surface samples and depth samples, showing the most significant differences in surface river samples (Sar, Herbón, Cesures, Bexo and Isorna), where Fe concentrations were markedly higher in 2002.

4 - Most values obtained in our study were higher than those previously reported by Prego and Cobelo-García [7] for typical Fe concentrations at the Galician Rías (from 1.95 to 8.93 μg L^{-1}). However, values presented in our work were in the same range than iron concentrations reported by other authors [7] for estuarine and coastal waters (from 0.11 to 93.8 μg L^{-1}). River water concentration was also in the same order reported by Filgueiras and Prego, ranging from 55.8 to 251 μg L^{-1} [16].

3.7. Aluminum

1 - In 2002, the Al concentration was between 8 and 37 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the mouth of the Ulla River) which exhibited higher concentrations of Al. The Al levels at these stations ranged between 55 and 86 μg L^{-1} for samples located at the mouth of the river, and from 83 to 155 μg L^{-1} for the river samples. The Al concentration in depth samples was similar to surface samples with levels ranging between 6 and 36 μg L^{-1} at all sampling points, except for 1, 22 and 24 stations (located at the inner part of the estuary, near the mouth of the Ulla River) with values between 52.5 and 82 μg L^{-1}.

2 - In 2003, the Al concentration was between 3.5 and 27.5 μg L^{-1} at all surface samples, except for the river stations (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22, 24 (located at the mouth of the Ulla River) with higher concentrations of Al. The Al levels at these stations ranged between 31 and 59 μg L^{-1} for samples located at the mouth of the river, and 40 and 114 μg L^{-1} for the river samples. In general, the Al concentration in depth samples was similar to surface samples, with levels ranging from 10.5 to 34 μg L^{-1} at all sampling points, except for stations 1, 22 and 24 (located at the inner part of the estuary, near the mouth of the Ulla River) with values between 37.5 and 50.5 μg L^{-1}.

3 - Generally, Al concentrations were similar in the two sampling campaigns (2002 and 2003), both in surface samples and depth samples, showing the most significant differences in surface river samples (Sar, Herbón, Cesures, Bexo and Isorna), where Al concentrations were markedly higher in 2002.

4 – No Al values at the Galician Rías were found in literature, so it was not possible to establish a comparison with previously reported Al concentrations.

3.8. Zinc

1 - In 2002, the Zn concentration ranged from 3 to 30.5 μg L^{-1} at all surface samples, except for stations 1 (located in front of "Vilagarcía Arousa"), 3 and 4 (located in the neighbourhood of the "Arousa Island") and 20 (located in front of "Pobra do Caramiñal") which exhibited higher concentrations ranging between 33 and 37 μg L^{-1}. In general, the Zn concentration in depth samples was slightly lower at most stations studied, with concentrations ranging from 1.5 to 20 μg L^{-1}.

2 - In 2003, the Zn concentration ranged between 1.5 and 16 μg L^{-1} at all surface samples, except for stations 1 (located in front of "Vilagarcía de Arousa") and 5, showing higher concentrations, ranging between 26.5 and 28 μg L^{-1}. In general, the Zn concentration in depth samples was similar to the Zn concentration in surface samples, with values between 1 and 22.5 μg L^{-1} at all stations studied.

3 - In general, Zn concentrations were similar in the two sampling campaigns (2002 and 2003), both in surface samples and depth samples.

4 - All values obtained in our study were in the same range than those previously reported by Prego and Cobelo-García [7] for typical Zn concentrations at the Galician Rías (from 2.29 to 22.4 μg L^{-1}).

4. Conclusions

From the results obtained, the following conclusions can be made:

4.1. First sampling campaign (2002)

1 - In general, Cu, Ni, Pb, Mn, Sn, Fe and Al concentrations in shallow waters were much higher in samples from Ulla River (Sar, Herbón, Cesures, Bexo and Isorna) and stations located at the mouth of the Ulla River (1, 22 and 24), than the other stations studied. Zn concentration in shallow waters was slightly higher at stations 1, 3, 4 and 20, located in the neighbourhood of "Vilagarcía Arousa", "Arousa Island" and "Pobra do Caramiñal", respectively.

2 - In general, Ni, Mn, Sn, Fe and Al levels were quite similar in both depth and surface samples. Cu and Pb concentrations were higher in depth samples whereas Zn concentration was higher in most of the surface samples studied.

3 - Figure 2 shows the results obtained after conducting a principal components analysis for 2002 surface samples. As shown in this figure, there are two different groups of samples. The first one is formed by the river samples (R1, R2, R3, R4 and R5) and the second one is formed by the estuary samples. Inside the second group, we can distinguish between samples 3, 4, 15 and 20 (belonging to stations located mainly at the middle estuary), samples 2, 5, 8, 18, 19, 21 and 22 (belonging to stations located mainly at the lower estuary) and samples 6, 7, 9, 11, 12, 13, 14 and 16 (belonging to stations located mainly at the upper estuary). Samples 1 and 24 (belonging to stations located at the mouth of the Ulla River) do not belong to any of the previous groups. The weights of each variable in the two first principal components are shown in Table 3. Principal component 1 (PC1) is formed by the variables Cu, Ni, Pb, Mn, Sn, Fe and Al while principal component 2 (PC2) is formed by the variable Zn. The two first principal components explained a percentage of variability of 93% (PC1 explained 81% whereas PC2 explains 12%). The values of the residuals were negligible for all the points, so all samples were considered in the study of PCA. Similar results were obtained after conducting a cluster analysis (Figure 3).

Figure 2. Principal components analysis for surface samples (2002).

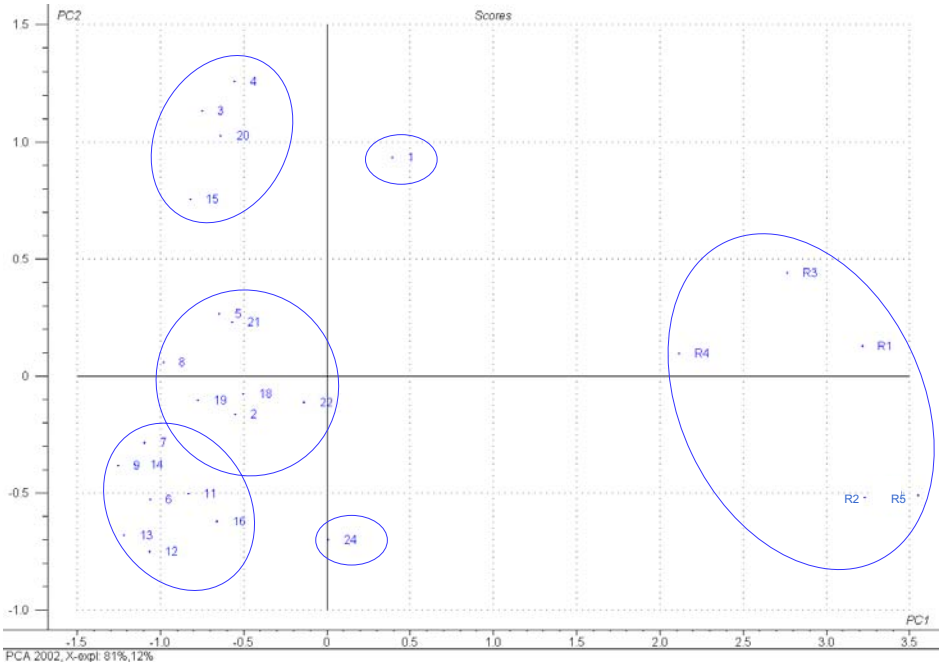
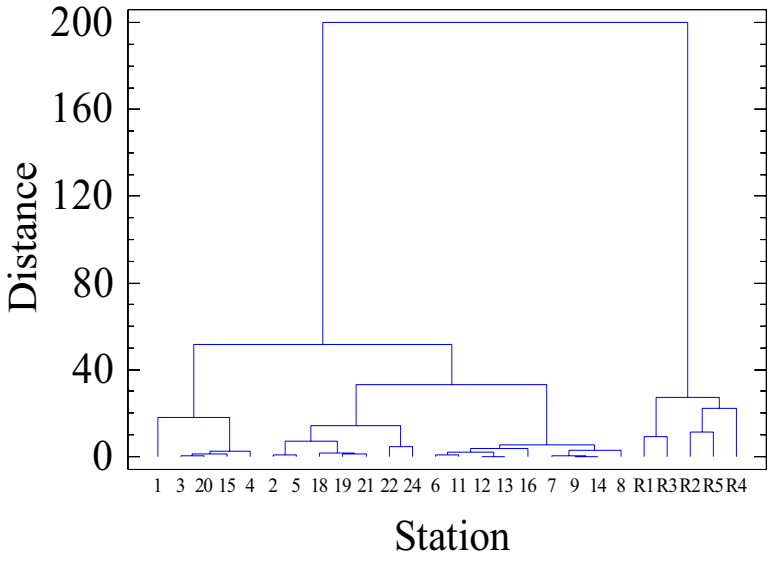


Table 3. Weight of each variable in the two first principal components (Shallow waters 2002)

VARIABLES	PC1	PC2
Cu	0.344	-0.008
Ni	0.285	0.058
Pb	0.344	-0.004
Mn	0.453	-0.071
Sn	0.407	-0.064
Fe	0.403	-0.089
Al	0.371	-0.059
Zn	0.105	0.988

Figure 3. Cluster analysis for surface samples (2002).



4.2. Second sampling campaign (2003)

1 - In general, Mn, Sn, Fe and Al concentrations in shallow waters were much higher in samples from Ulla River (Sar, Herbón, Cesures, Bexo and Isorna) and stations 1, 22 and 24 (located at the mouth of the Ulla River), than the other stations studied. Cu, Ni, Pb and Zn concentrations were similar for all surface samples studied, founding slightly higher levels of Cu and Pb in front of "Vilagarcia de Arousa" and "Boiro", slightly higher levels of Ni in front of "O Grove" and "Laxe", and slightly higher levels of Zn in front of "Vilagarcia de Arousa" and the 5th station.

2 – In general, Cu, Ni, Pb, Sn, Al, Fe and Zn levels were quite similar in both depth and surface samples, while the Mn concentration was higher in surface samples at most of the stations studied.

3 - Figure 4 shows the results obtained after conducting a principal components analysis for 2003 surface samples. As shown in this figure, there are four different groups of samples. The first one is formed by the river samples (R1, R2, R3, R4 and R5), the second group is formed by samples 1, 22 and 24 (belonging to stations located at the mouth of the Ulla River), the third group is formed by samples 2, 8, 12, 14, 16 and 20 (belonging to stations located mainly at the outer part of the Ría, near the estuary shores), and the fourth group is formed by samples 3, 4, 5, 6, 7, 9, 11, 13, 15, 18 and 19 (belonging to stations located mainly at the inner part of the Ría). The weights of each variable in the two first principal components are shown in Table 4. Principal component 1 (PC1) is formed by the variables Mn, Sn, Fe and Al while principal component 2 (PC2) is formed by the variables Cu, Ni, Pb, Zn. The two first principal components explained a percentage of variability of 77% (PC1 explained 51% whereas PC2 explains 26%). The values of the residuals were negligible for all the points, so all samples were considered in the study of PCA. Similar results were obtained after conducting a cluster analysis (Figure 5).

4.3. Comparison between the first and the second sampling campaign.

1 - In general, Cu, Ni, Pb and Mn levels at shallow waters were higher in 2003. This trend was reversed when dealing with river waters, where metal concentrations were markedly higher in 2002. Sn, Fe, Al and Zn concentrations in shallow waters were similar at both sampling campaigns, being the most significant differences at river samples (Sar, Herbón, Cesures, Bexo and Isorna) where these metal concentrations were markedly higher in 2002.

2 - In general, Ni, Mn, Sn, Fe, Al and Zn concentrations in depth samples were quite similar at both sampling campaigns (2002 and 2003), whereas Cu and Pb concentrations were slightly higher in 2002 at most of the stations studied.

Figure 4. Principal components analysis for surface samples (2003).

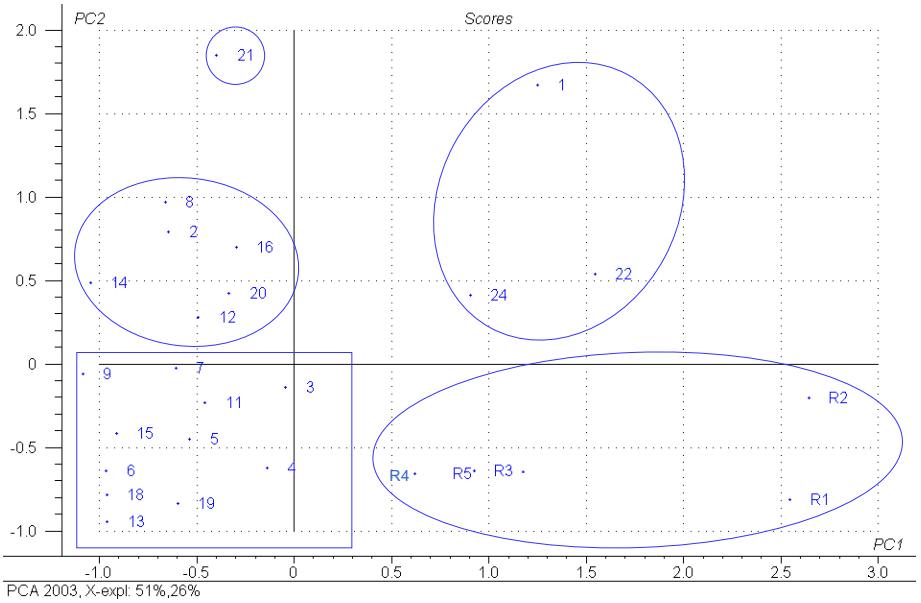
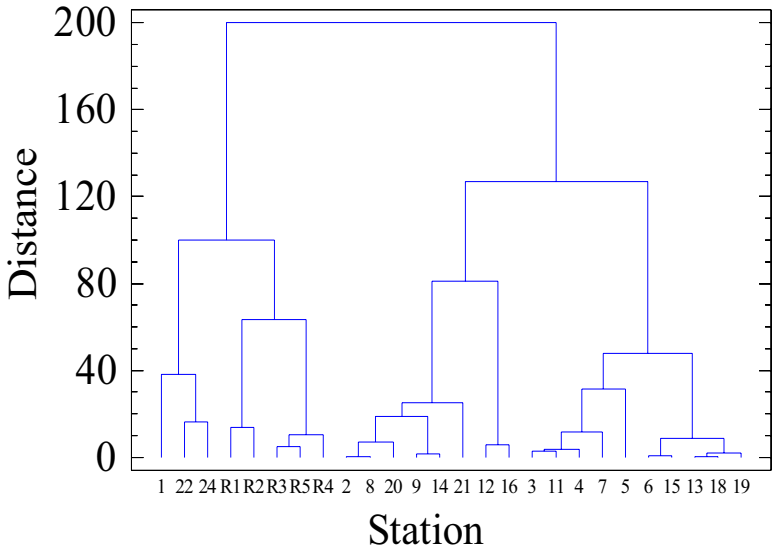


Table 4. Weight of each variable in the two first principal components (Shallow waters 2003)

VARIABLES	PC1	PC2
Cu	0.019	0.674
Ni	0.057	0.181
Pb	0.032	0.701
Mn	0.530	0.029
Sn	0.508	-0.056
Fe	0.479	-0.057
Al	0.469	-0.032
Zn	0.082	0.119

Figure 5. Cluster analysis for surface samples (2003).



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Supplementary data

Table S1. Copper concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St. Code	Surface 2002	Surface 2003	Depth /m	Mid-depth 2002	Mid-depth 2003	Depth /m	Near estuary bed 2002	Near estuary bed 2003
1	1.40 ± 0.09	7.84 ± 0.11	5	5.97 ± 1.14	2.26 ± 0.23			
2	2.08 ± 0.02	5.71 ± 1.00	15	5.73 ± 0.58	4.27 ± 0.32			
3	0.93 ± 0.19	2.88 ± 0.58	20	6.41 ± 0.21	3.46 ± 0.41			
4	1.01 ± 0.23	1.54 ± 0.12	20	8.56 ± 1.28	2.63 ± 0.08	40	3.02 ± 0.60	2.58 ± 0.09
5	1.93 ± 0.01	1.13 ± 0.21	25	5.33 ± 0.13	3.73 ± 0.75	50	5.14 ± 0.05	2.27 ± 0.11
6	0.17 ± 0.14	1.61 ± 0.32	20	4.13 ± 0.18	2.66 ± 0.53	40	4.51 ± 0.57	2.50 ± 0.34
7	0.28 ± 0.08	2.92 ± 0.58	20	3.65 ± 0.32	2.37 ± 0.04			
8	0.27 ± 0.04	6.11 ± 0.53	30	2.96 ± 0.59	2.38 ± 0.26			
9	0.34 ± 0.23	3.15 ± 0.29	15	5.54 ± 0.73	4.76 ± 0.53			
11	0.44 ± 0.05	2.64 ± 0.07	5	4.61 ± 0.92	3.00 ± 0.10			
12	0.50 ± 0.17	3.46 ± 0.28	15	2.92 ± 0.50	2.65 ± 0.46			
13	0.53 ± 0.11	0.69 ± 0.03	30	3.33 ± 0.12	1.99 ± 0.29			
14	0.45 ± 0.02	4.80 ± 0.60	30	2.03 ± 0.16	2.44 ± 0.41			
15	0.51 ± 0.01	2.13 ± 0.43	20	4.65 ± 0.93	1.96 ± 0.11			
16	1.36 ± 0.27	4.79 ± 0.37	30	3.65 ± 0.65	1.03 ± 0.21			
18	1.31 ± 0.26	1.24 ± 0.13	10	6.08 ± 0.95	2.10 ± 0.31			
19	0.53 ± 0.20	1.10 ± 0.05	30	8.65 ± 0.52	1.44 ± 0.09			
20	0.58 ± 0.04	4.58 ± 0.59	10	5.70 ± 0.67	1.35 ± 0.13			
21	0.66 ± 0.12	8.99 ± 1.80	15	3.80 ± 0.57	2.66 ± 0.29			
22	0.67 ± 0.11	4.69 ± 0.94	10	5.42 ± 0.79	1.17 ± 0.21			
24	1.14 ± 0.19	4.85 ± 0.42	5	7.59 ± 1.55	2.26 ± 0.35			
	River (2002)	River (2003)						
R1	6.29 ± 0.16	1.39 ± 0.11						
R2	5.51 ± 0.09	2.95 ± 0.28						
R3	4.55 ± 0.81	1.82 ± 0.11						
R4	3.04 ± 0.21	1.77 ± 0.35						
R5	4.16 ± 0.26	1.57 ± 0.15						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S2. Nickel concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St.	Surface		Depth	Mid-depth		Depth	Near estuary bed	
Code	2002	2003	/m	2002	2003	/m	2002	2003
1	0.81 ± 0.16	1.42 ± 0.08	5	1.27 ± 0.29	0.40 ± 0.04			
2	0.53 ± 0.11	1.07 ± 0.21	15	1.15 ± 0.23	1.08 ± 0.05			
3	0.58 ± 0.12	1.17 ± 0.23	20	1.04 ± 0.03	0.32 ± 0.07			
4	1.42 ± 0.28	0.72 ± 0.04	20	0.50 ± 0.09	0.51 ± 0.10	40	0.31 ± 0.05	0.66 ± 0.12
5	0.46 ± 0.12	1.97 ± 0.12	25	0.34 ± 0.06	0.76 ± 0.15	50	0.50 ± 0.10	0.59 ± 0.06
6	0.50 ± 0.10	1.00 ± 0.20	20	0.48 ± 0.10	0.30 ± 0.03	40	0.76 ± 0.01	0.65 ± 0.13
7	0.29 ± 0.04	1.85 ± 0.37	20	0.48 ± 0.11	0.84 ± 0.18			
8	0.34 ± 0.02	1.37 ± 0.07	30	0.61 ± 0.07	0.32 ± 0.02			
9	0.28 ± 0.07	0.50 ± 0.04	15	0.28 ± 0.06	1.21 ± 0.24			
11	0.41 ± 0.01	0.59 ± 0.12	5	0.64 ± 0.11	0.70 ± 0.08			
12	0.18 ± 0.04	4.24 ± 0.16	15	0.91 ± 0.08	0.25 ± 0.05			
13	0.32 ± 0.06	0.31 ± 0.01	30	0.39 ± 0.08	0.36 ± 0.04			
14	0.34 ± 0.11	0.65 ± 0.06	30	0.44 ± 0.09	0.38 ± 0.01			
15	0.92 ± 0.18	0.77 ± 0.15	20	0.72 ± 0.14	0.44 ± 0.04			
16	0.47 ± 0.10	3.34 ± 0.67	30	0.87 ± 0.01	0.42 ± 0.06			
18	1.38 ± 0.18	0.69 ± 0.06	10	0.54 ± 0.11	0.47 ± 0.07			
19	0.97 ± 0.03	0.66 ± 0.07	30	1.77 ± 0.35	0.43 ± 0.07			
20	0.92 ± 0.12	1.02 ± 0.12	10	1.13 ± 0.23	0.34 ± 0.05			
21	1.39 ± 0.28	1.16 ± 0.05	15	2.05 ± 0.41	0.30 ± 0.01			
22	1.34 ± 0.08	2.09 ± 0.42	10	1.37 ± 0.02	0.35 ± 0.05			
24	0.72 ± 0.01	1.05 ± 0.06	5	1.40 ± 0.28	0.46 ± 0.02			
	River (2002)	River (2003)						
R1	1.83 ± 0.31	0.99 ± 0.20						
R2	4.32 ± 0.37	1.73 ± 0.11						
R3	2.50 ± 0.09	1.23 ± 0.11						
R4	3.48 ± 0.15	0.94 ± 0.19						
R5	2.48 ± 0.19	1.05 ± 0.06						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S3. Lead concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St.	Surface		Depth	Mid-depth		Depth	Near estuary bed	
Code	2002	2003	/m	2002	2003	/m	2002	2003
1	1.52 ± 0.04	7.67 ± 0.12	5	6.13 ± 1.00	2.15 ± 0.17			
2	2.14 ± 0.11	5.52 ± 0.95	15	5.96 ± 0.75	4.34 ± 0.37			
3	1.00 ± 0.20	2.85 ± 0.57	20	6.33 ± 0.24	3.39 ± 0.43			
4	1.13 ± 0.12	1.32 ± 0.01	20	8.44 ± 1.19	2.60 ± 0.07	40	2.95 ± 0.59	2.50 ± 0.12
5	1.94 ± 0.07	1.16 ± 0.23	25	5.21 ± 0.12	3.65 ± 0.73	50	5.16 ± 0.10	2.22 ± 0.13
6	0.20 ± 0.04	1.28 ± 0.26	20	4.09 ± 0.23	2.89 ± 0.30	40	4.42 ± 0.60	2.41 ± 0.40
7	0.49 ± 0.10	2.95 ± 0.59	20	3.54 ± 0.38	2.31 ± 0.08			
8	0.34 ± 0.06	5.90 ± 0.30	30	2.85 ± 0.57	2.41 ± 0.35			
9	0.43 ± 0.09	3.13 ± 0.10	15	4.33 ± 0.82	4.80 ± 0.58			
11	0.32 ± 0.06	2.75 ± 0.02	5	4.62 ± 0.84	3.02 ± 0.22			
12	0.48 ± 0.01	3.24 ± 0.21	15	2.62 ± 0.10	2.55 ± 0.50			
13	0.50 ± 0.10	0.68 ± 0.10	30	3.31 ± 0.27	2.06 ± 0.41			
14	0.49 ± 0.05	4.65 ± 0.66	30	2.07 ± 0.10	2.47 ± 0.45			
15	0.69 ± 0.10	2.02 ± 0.40	20	5.46 ± 1.28	1.94 ± 0.12			
16	1.41 ± 0.28	4.74 ± 0.28	30	3.59 ± 0.71	1.11 ± 0.20			
18	1.38 ± 0.28	1.00 ± 0.12	10	6.09 ± 1.04	1.98 ± 0.29			
19	0.82 ± 0.16	1.00 ± 0.02	30	8.60 ± 0.60	1.34 ± 0.11			
20	0.72 ± 0.08	4.29 ± 0.60	10	5.52 ± 0.65	1.23 ± 0.14			
21	0.73 ± 0.11	8.35 ± 1.67	15	3.72 ± 0.55	2.54 ± 0.34			
22	0.77 ± 0.10	4.48 ± 0.90	10	5.38 ± 0.84	1.15 ± 0.20			
24	1.32 ± 0.10	4.61 ± 0.42	5	7.60 ± 1.58	2.34 ± 0.38			
	River (2002)	River (2003)						
R1	6.64 ± 0.17	1.45 ± 0.11						
R2	5.80 ± 0.21	3.08 ± 0.41						
R3	4.65 ± 0.82	1.81 ± 0.15						
R4	3.19 ± 0.28	1.57 ± 0.31						
R5	4.62 ± 0.27	1.55 ± 0.08						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S4. Manganese concentrations in the Ría de Arousa and the Ulla River ($\mu g\ L^{-1}$)

St. Code	Surface 2002	Surface 2003	Depth /m	Mid-depth 2002	Mid-depth 2003	Depth /m	Near estuary bed 2002	Near estuary bed 2003
1	7.08 ± 0.17	17.24 ± 0.33	5	4.69 ± 0.81	2.02 ± 0.31			
2	2.71 ± 0.07	2.48 ± 0.71	15	2.74 ± 0.41	1.75 ± 0.19			
3	1.58 ± 0.27	8.43 ± 1.69	20	1.61 ± 0.38	1.08 ± 0.17			
4	2.48 ± 0.26	9.28 ± 0.12	20	1.55 ± 0.26	0.89 ± 0.18	40	1.68 ± 0.04	0.90 ± 0.17
5	2.32 ± 0.05	6.84 ± 0.81	25	1.72 ± 0.20	0.61 ± 0.12	50	1.48 ± 0.16	0.85 ± 0.10
6	0.95 ± 0.04	1.89 ± 0.05	20	0.87 ± 0.15	0.35 ± 0.05	40	0.67 ± 0.11	0.32 ± 0.06
7	0.67 ± 0.06	2.19 ± 0.44	20	0.92 ± 0.02	0.43 ± 0.09			
8	1.31 ± 0.26	5.15 ± 0.42	30	0.98 ± 0.04	0.65 ± 0.09			
9	0.84 ± 0.17	2.58 ± 0.08	15	0.59 ± 0.01	1.29 ± 0.18			
11	1.40 ± 0.23	2.33 ± 0.47	5	1.56 ± 0.32	1.61 ± 0.39			
12	1.32 ± 0.26	5.67 ± 0.07	15	1.01 ± 0.03	0.51 ± 0.12			
13	0.78 ± 0.05	4.16 ± 0.21	30	0.82 ± 0.15	0.61 ± 0.03			
14	1.44 ± 0.07	2.17 ± 0.43	30	1.22 ± 0.14	0.86 ± 0.17			
15	2.13 ± 0.15	1.87 ± 0.28	20	1.12 ± 0.08	1.20 ± 0.19			
16	3.47 ± 0.16	8.28 ± 1.66	30	0.77 ± 0.06	0.46 ± 0.07			
18	2.77 ± 0.14	5.04 ± 0.76	10	1.88 ± 0.21	0.77 ± 0.07			
19	3.03 ± 0.09	8.80 ± 0.38	30	2.14 ± 0.27	0.36 ± 0.01			
20	2.44 ± 0.11	8.48 ± 0.92	10	2.29 ± 0.03	0.38 ± 0.02			
21	2.46 ± 0.26	8.24 ± 0.81	15	1.69 ± 0.31	1.13 ± 0.03			
22	6.11 ± 0.89	22.99 ± 0.05	10	3.72 ± 0.17	1.75 ± 0.25			
24	8.53 ± 0.92	14.26 ± 2.27	5	6.58 ± 0.32	4.69 ± 0.94			
	River (2002)	River (2003)						
R1	31.32 ± 1.19	19.31 ± 0.44						
R2	37.22 ± 2.55	24.09 ± 2.26						
R3	37.21 ± 2.71	13.29 ± 1.86						
R4	31.55 ± 1.26	11.34 ± 2.27						
R5	37.88 ± 1.02	18.18 ± 0.64						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S5. Tin concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St.	Surface		Depth	Mid-depth		Depth	Near estuary bed	
Code	2002	2003	/m	2002	2003	/m	2002	2003
1	20.91 ± 2.25	16.22 ± 0.44	5	20.81 ± 2.75	11.61 ± 0.08			
2	5.81 ± 0.02	4.70 ± 0.69	15	8.35 ± 1.64	2.98 ± 0.32			
3	4.36 ± 0.10	9.68 ± 1.94	20	3.71 ± 0.09	7.94 ± 0.93			
4	5.08 ± 0.08	7.47 ± 1.00	20	2.55 ± 0.04	8.33 ± 0.02	40	3.85 ± 0.88	10.89 ± 0.93
5	4.76 ± 0.12	3.24 ± 0.18	25	3.70 ± 0.28	2.07 ± 0.41	50	10.81 ± 0.46	6.31 ± 0.71
6	6.97 ± 0.49	3.50 ± 0.51	20	2.46 ± 0.21	2.43 ± 0.22	40	2.97 ± 0.40	3.23 ± 0.53
7	5.59 ± 0.13	6.15 ± 0.30	20	1.25 ± 0.11	5.50 ± 1.09			
8	6.92 ± 0.73	3.64 ± 0.17	30	4.33 ± 0.90	10.97 ± 0.73			
9	2.26 ± 0.01	1.28 ± 0.04	15	2.25 ± 0.16	4.42 ± 0.92			
11	8.52 ± 0.25	6.87 ± 1.37	5	6.75 ± 0.31	3.52 ± 0.90			
12	5.17 ± 0.30	5.16 ± 0.20	15	3.89 ± 0.52	2.72 ± 0.54			
13	2.98 ± 0.17	1.58 ± 0.16	30	4.37 ± 0.24	4.40 ± 0.04			
14	3.15 ± 0.15	1.32 ± 0.26	30	7.95 ± 0.31	7.62 ± 0.35			
15	4.76 ± 0.11	2.67 ± 0.53	20	2.38 ± 0.11	8.11 ± 0.63			
16	8.19 ± 1.00	5.36 ± 0.43	30	6.51 ± 0.92	2.89 ± 0.24			
18	9.91 ± 0.43	1.73 ± 0.13	10	4.22 ± 0.32	2.61 ± 0.42			
19	6.06 ± 0.19	3.23 ± 0.05	30	7.31 ± 0.41	6.94 ± 1.28			
20	6.66 ± 0.04	4.43 ± 0.37	10	2.16 ± 0.38	4.15 ± 0.50			
21	8.43 ± 0.95	3.31 ± 0.18	15	7.29 ± 0.69	1.86 ± 0.08			
22	14.42 ± 1.07	15.63 ± 0.94	10	12.64 ± 0.36	14.42 ± 0.38			
24	16.39 ± 1.99	16.44 ± 1.11	5	17.90 ± 0.90	11.71 ± 0.89			
	River (2002)	River (2003)						
R1	40.96 ± 0.87	27.47 ± 1.88						
R2	39.88 ± 4.00	24.46 ± 0.12						
R3	37.19 ± 3.24	13.56 ± 1.20						
R4	25.49 ± 2.35	19.47 ± 1.89						
R5	42.80 ± 3.55	10.87 ± 2.17						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S6. Iron concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St.	Surface		Depth	Mid-depth		Depth	Near estuary bed	
Code	2002	2003	/m	2002	2003	/m	2002	2003
1	78.99 ± 3.33	75.62 ± 2.70	5	65.48 ± 4.72	55.07 ± 1.53			
2	28.42 ± 2.52	26.83 ± 1.99	15	24.59 ± 3.48	25.40 ± 1.40			
3	22.44 ± 0.03	44.89 ± 3.98	20	22.02 ± 1.87	44.83 ± 4.64			
4	24.63 ± 0.51	43.51 ± 4.23	20	16.25 ± 0.46	46.58 ± 0.08	40	16.27 ± 0.74	25.45 ± 1.62
5	22.43 ± 2.48	18.12 ± 0.91	25	22.70 ± 1.74	19.81 ± 3.96	50	21.88 ± 2.57	26.66 ± 1.69
6	37.09 ± 2.67	20.25 ± 2.12	20	15.64 ± 0.57	15.32 ± 1.07	40	18.52 ± 1.70	19.94 ± 1.05
7	30.11 ± 1.64	37.51 ± 1.55	20	9.56 ± 0.04	31.72 ± 6.19			
8	35.75 ± 6.14	21.02 ± 0.44	30	26.56 ± 4.66	22.21 ± 1.62			
9	11.03 ± 0.42	9.73 ± 0.12	15	14.34 ± 0.40	8.77 ± 0.33			
11	43.92 ± 1.28	49.29 ± 9.74	5	38.92 ± 2.04	35.77 ± 1.31			
12	30.82 ± 3.98	29.31 ± 0.80	15	23.23 ± 2.93	16.36 ± 3.27			
13	14.04 ± 0.15	13.03 ± 2.35	30	26.57 ± 1.70	25.99 ± 0.07			
14	16.47 ± 0.43	11.43 ± 1.87	30	16.62 ± 1.02	14.52 ± 1.10			
15	25.52 ± 1.53	16.59 ± 3.32	20	15.57 ± 0.45	15.88 ± 1.78			
16	44.55 ± 5.34	30.24 ± 3.15	30	39.96 ± 4.38	18.12 ± 0.32			
18	25.50 ± 1.60	9.63 ± 0.61	10	25.60 ± 2.44	18.76 ± 3.77			
19	31.60 ± 1.83	19.18 ± 0.75	30	22.52 ± 2.64	23.71 ± 8.69			
20	33.90 ± 0.58	26.62 ± 2.21	10	14.44 ± 1.29	24.61 ± 2.29			
21	43.10 ± 5.04	20.08 ± 1.42	15	41.39 ± 2.82	13.85 ± 0.08			
22	75.15 ± 6.49	87.45 ± 5.35	10	69.98 ± 2.45	66.10 ± 3.05			
24	71.51 ± 9.38	76.33 ± 2.32	5	69.59 ± 4.34	66.38 ± 5.49			
	River (2002)	River (2003)						
R1	207.28 ± 2.10	150.10 ± 11.22						
R2	209.30 ± 22.23	132.49 ± 0.80						
R3	193.72 ± 16.89	87.19 ± 8.67						
R4	131.94 ± 13.22	52.13 ± 10.43						
R5	223.19 ± 19.05	58.97 ± 11.79						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S7. Aluminium concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St. Code	Surface 2002	Surface 2003	Depth /m	Mid-depth 2002	Mid-depth 2003	Depth /m	Near estuary bed 2002	Near estuary bed 2003
1	85.94 ± 11.38	51.60 ± 1.49	5	81.60 ± 2.38	48.61 ± 0.55			
2	23.62 ± 0.74	22.15 ± 0.76	15	25.77 ± 1.72	26.46 ± 1.87			
3	21.88 ± 0.11	23.97 ± 4.79	20	16.92 ± 1.56	31.12 ± 2.54			
4	22.10 ± 1.10	19.56 ± 0.96	20	12.90 ± 0.33	33.72 ± 4.74	40	11.84 ± 1.35	38.76 ± 1.53
5	16.19 ± 1.41	12.99 ± 0.83	25	17.91 ± 1.57	13.89 ± 0.03	50	18.91 ± 2.35	23.06 ± 0.83
6	12.21 ± 0.87	5.42 ± 1.08	20	11.68 ± 0.96	10.91 ± 1.88	40	14.01 ± 2.45	12.50 ± 2.18
7	10.27 ± 0.89	10.80 ± 0.87	20	6.30 ± 0.35	24.11 ± 2.33			
8	14.43 ± 1.49	13.57 ± 1.52	30	18.40 ± 3.02	29.37 ± 3.03			
9	8.05 ± 0.38	3.53 ± 0.71	15	10.08 ± 1.28	3.37 ± 4.34			
11	36.55 ± 2.25	18.78 ± 0.15	5	38.00 ± 2.39	22.12 ± 2.88			
12	21.59 ± 0.50	13.53 ± 1.22	15	18.50 ± 0.64	13.25 ± 0.80			
13	10.01 ± 1.01	10.99 ± 0.31	30	18.99 ± 0.99	16.67 ± 0.28			
14	11.83 ± 0.89	6.85 ± 0.99	30	17.12 ± 2.53	22.15 ± 3.76			
15	15.85 ± 0.45	16.09 ± 2.35	20	11.35 ± 1.70	29.87 ± 1.31			
16	19.37 ± 1.62	19.79 ± 2.25	30	11.03 ± 2.11	13.45 ± 0.83			
18	27.44 ± 1.14	8.29 ± 0.71	10	20.10 ± 1.05	14.63 ± 2.47			
19	24.24 ± 3.21	17.08 ± 0.91	30	35.59 ± 2.46	29.66 ± 4.22			
20	28.48 ± 5.70	26.01 ± 0.57	10	12.06 ± 1.29	16.70 ± 1.21			
21	29.86 ± 2.60	27.50 ± 4.59	15	34.93 ± 3.15	10.69 ± 0.35			
22	55.47 ± 6.05	58.18 ± 0.36	10	52.56 ± 2.67	50.50 ± 2.62			
24	74.03 ± 7.27	46.21 ± 6.53	5	81.97 ± 6.56	37.66 ± 5.14			
	River (2002)	River (2003)						
R1	117.77 ± 3.47	103.10 ± 7.79						
R2	140.00 ± 13.87	113.83 ± 1.17						
R3	83.05 ± 15.57	66.79 ± 9.35						
R4	121.53 ± 9.81	40.18 ± 8.04						
R5	154.54 ± 9.34	48.20 ± 9.64						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Table S8. Zinc concentrations in the Ría de Arousa and the Ulla River (μg L^{-1})

St.	Surface		Depth	Mid-depth		Depth	Near estuary bed	
Code	2002	2003	/m	2002	2003	/m	2002	2003
1	34.63 ± 2.25	26.96 ± 5.39	5	19.34 ± 1.99	10.95 ± 2.25			
2	13.84 ± 2.21	7.73 ± 1.55	15	19.66 ± 4.22	5.57 ± 0.03			
3	35.14 ± 7.03	10.70 ± 0.34	20	9.67 ± 1.93	1.53 ± 0.30			
4	36.98 ± 7.40	15.22 ± 3.04	20	6.06 ± 1.21	1.14 ± 0.23	40	2.44 ± 0.49	8.85 ± 1.77
5	20.78 ± 4.16	27.80 ± 1.37	25	9.20 ± 1.84	22.18 ± 0.32	50	12.27 ± 2.45	18.96 ± 3.79
6	7.54 ± 0.11	7.47 ± 1.49	20	2.85 ± 0.65	5.82 ± 1.16	40	0.67 ± 0.13	8.90 ± 1.91
7	11.52 ± 1.39	12.47 ± 2.49	20	2.82 ± 0.31	3.87 ± 0.32			
8	17.45 ± 3.06	7.02 ± 0.43	30	2.52 ± 0.50	5.50 ± 0.33			
9	9.46 ± 0.87	9.26 ± 1.85	15	5.90 ± 1.18	14.67 ± 2.53			
11	8.55 ± 0.53	13.49 ± 2.70	5	6.63 ± 1.33	8.99 ± 1.26			
12	3.95 ± 0.79	4.17 ± 0.04	15	4.16 ± 0.83	7.10 ± 1.42			
13	4.53 ± 0.91	7.65 ± 0.91	30	2.85 ± 0.57	6.56 ± 1.31			
14	9.68 ± 1.94	8.06 ± 1.61	30	11.94 ± 2.39	5.75 ± 0.61			
15	28.65 ± 2.23	10.01 ± 2.00	20	1.68 ± 0.02	3.40 ± 0.27			
16	6.49 ± 0.50	5.67 ± 1.13	30	3.86 ± 0.43	2.57 ± 0.51			
18	15.08 ± 0.04	4.68 ± 0.94	10	7.79 ± 1.56	1.46 ± 0.29			
19	14.62 ± 0.57	2.66 ± 0.53	30	12.20 ± 0.44	2.90 ± 0.58			
20	33.65 ± 1.45	10.93 ± 1.90	10	3.70 ± 0.74	4.04 ± 0.81			
21	20.35 ± 0.96	5.22 ± 0.45	15	6.38 ± 1.28	2.36 ± 0.05			
22	15.95 ± 3.19	15.84 ± 3.17	10	7.62 ± 1.16	4.35 ± 0.87			
24	6.95 ± 0.82	7.98 ± 0.26	5	2.67 ± 0.13	5.08 ± 0.01			
	River (2002)	River (2003)						
R1	25.83 ± 1.93	12.82 ± 0.73						
R2	14.61 ± 2.61	8.16 ± 1.63						
R3	30.09 ± 0.58	1.83 ± 0.37						
R4	22.50 ± 0.38	7.06 ± 1.41						
R5	15.79 ± 1.55	4.78 ± 0.96						

R1 = Sar, R2 = Herbón, R3 = Cesures, R4 = Bexo, R5 = Isorna.

Figure S1a. Cupper isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

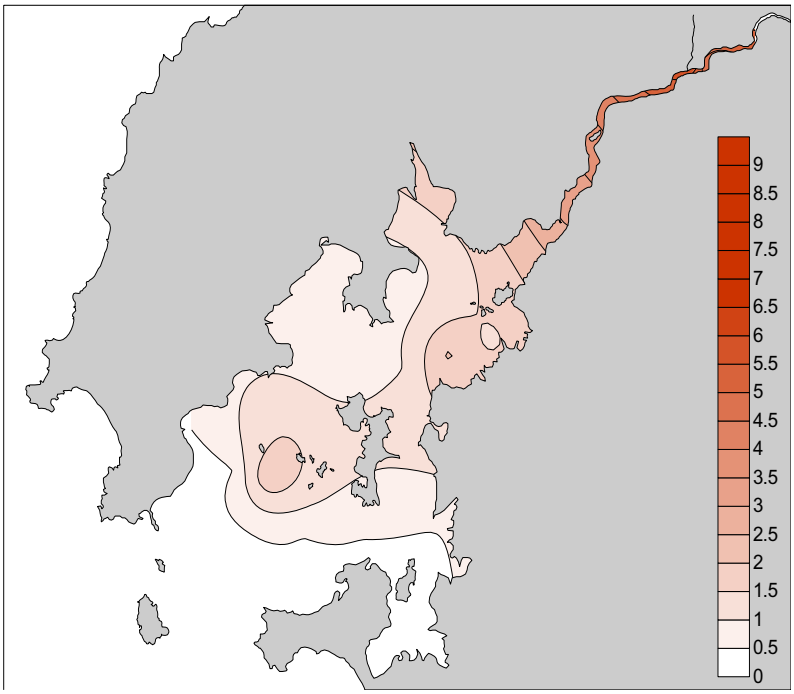


Figure S1b. Cupper isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

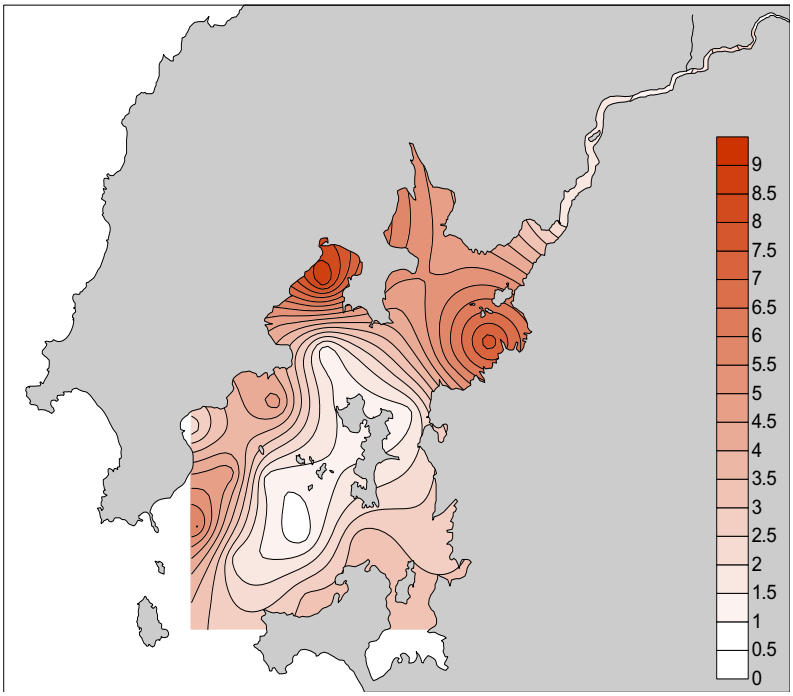


Figure S2a. Nickel isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

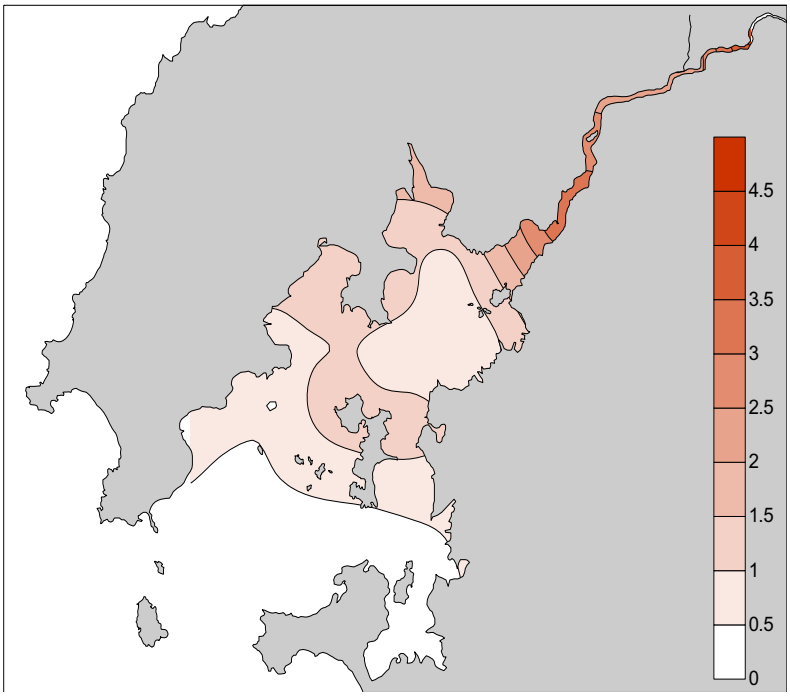


Figure S2b. Nickel isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

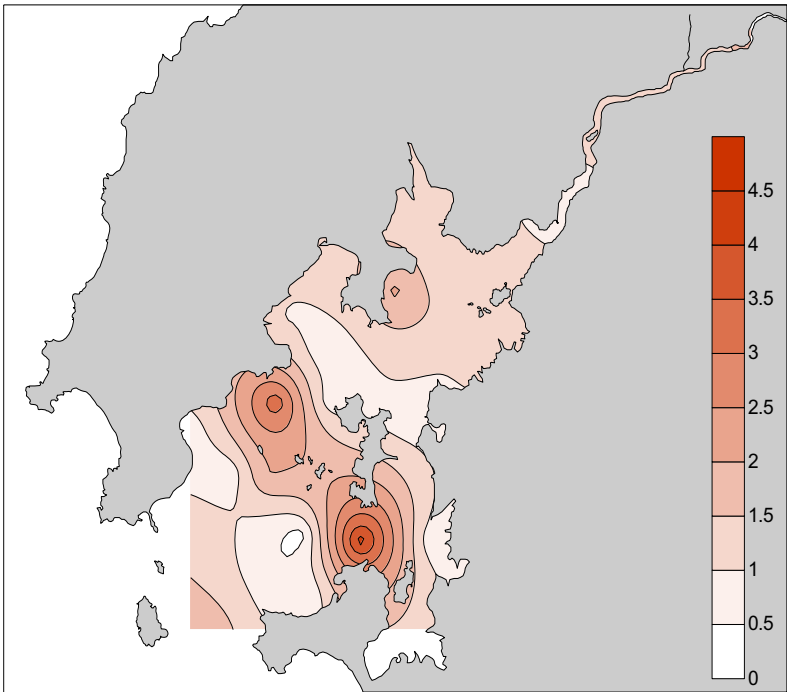


Figure S3a. Lead isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

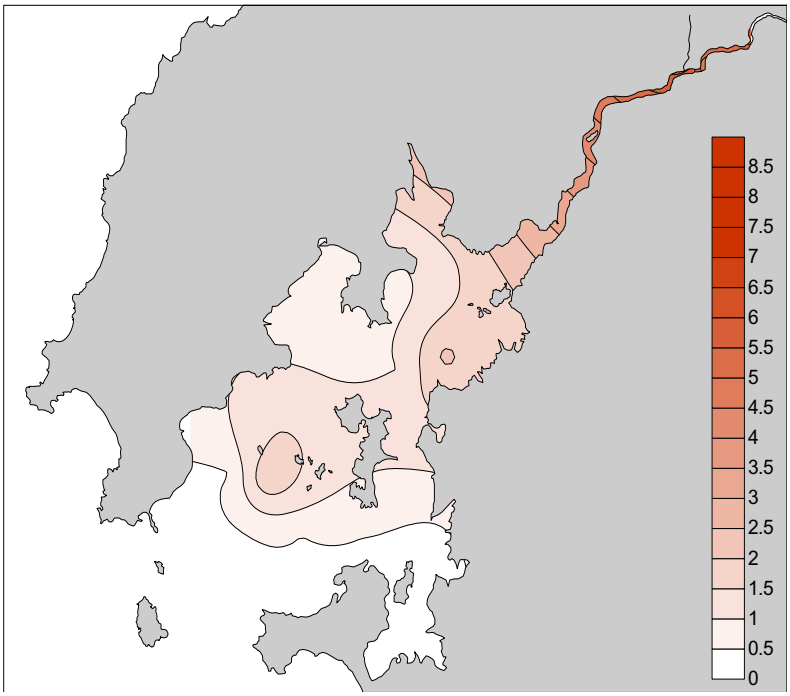


Figure S3b. Lead isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

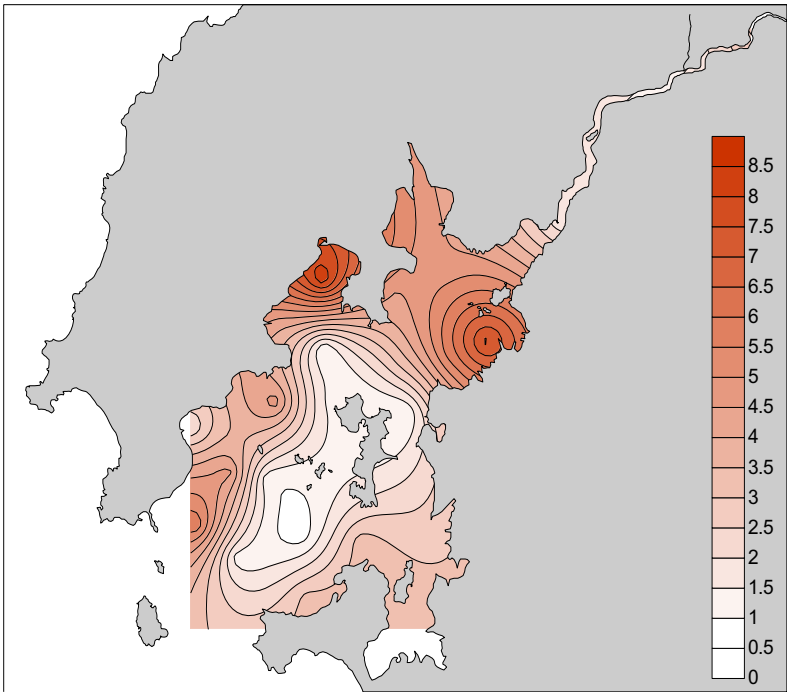


Figure S4a. Manganese isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

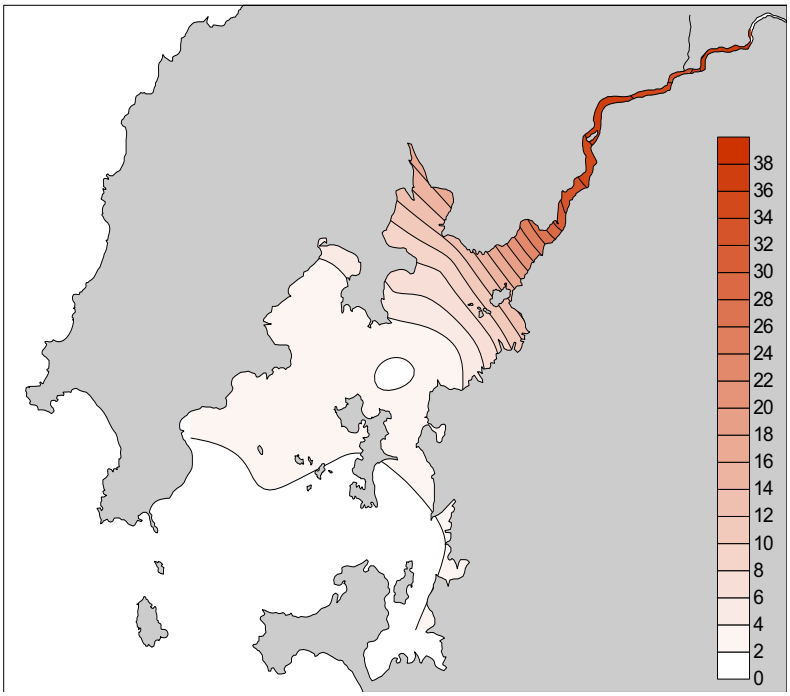


Figure S4b. Manganese isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

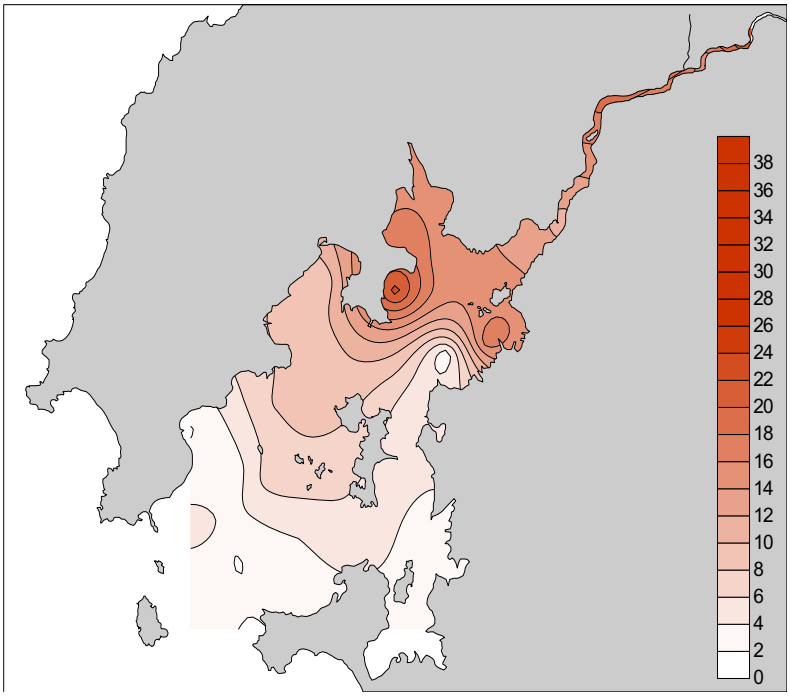


Figure S5a. Tin isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

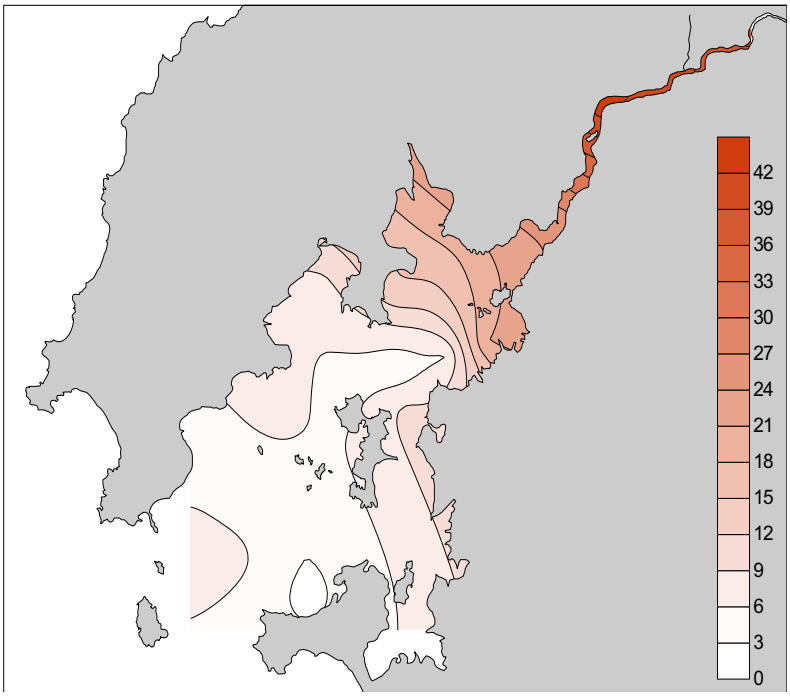


Figure S5b. Tin isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

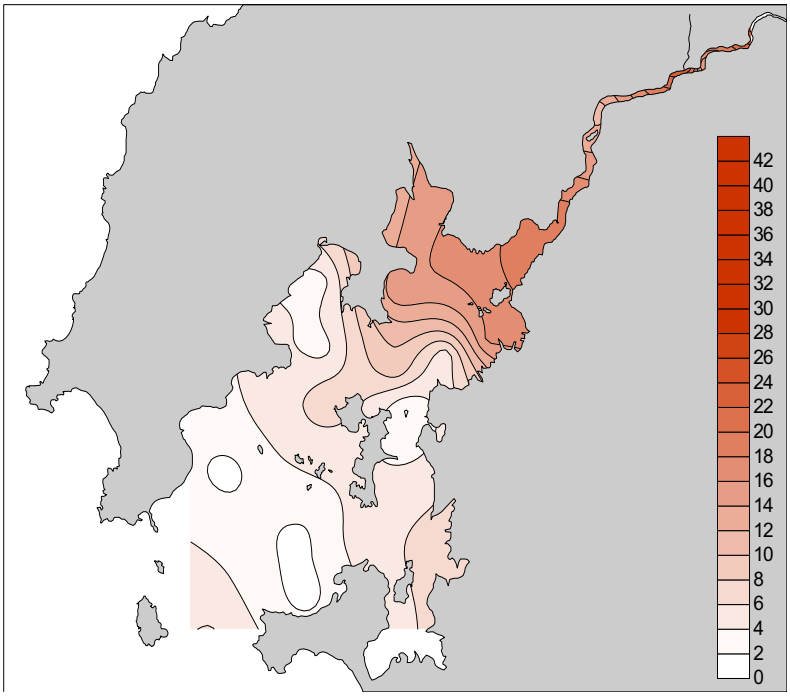


Figure S6a. Iron isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

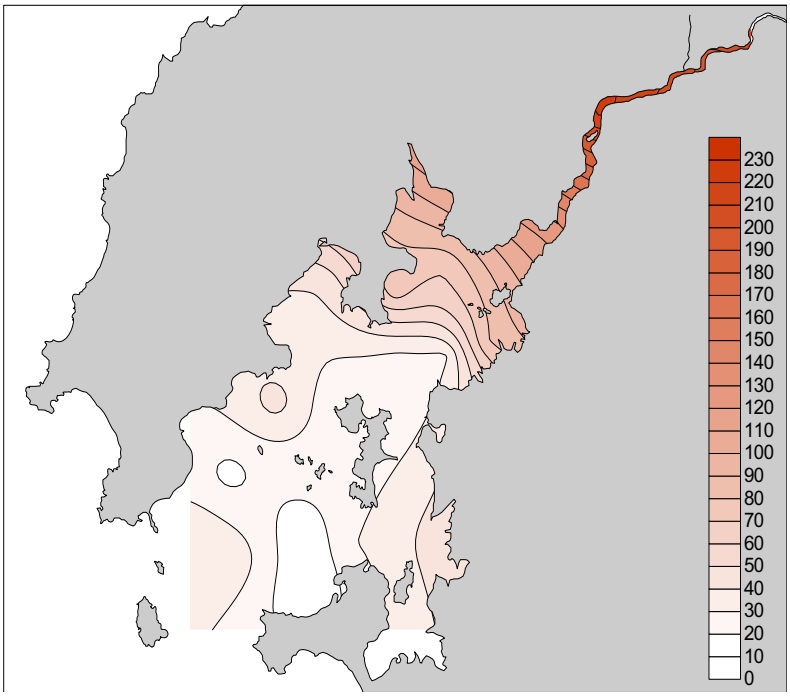


Figure S6b. Iron isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

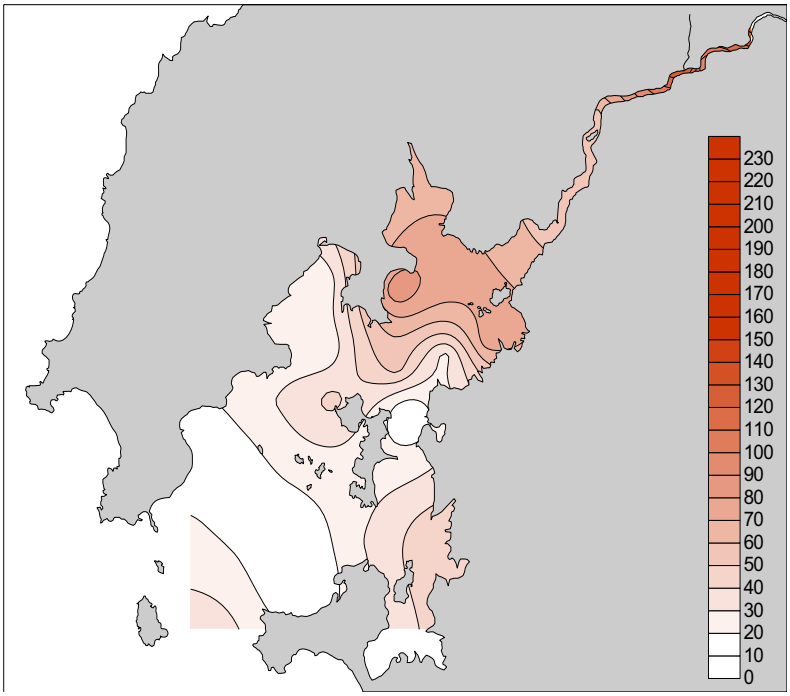


Figure S7a. Aluminium isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

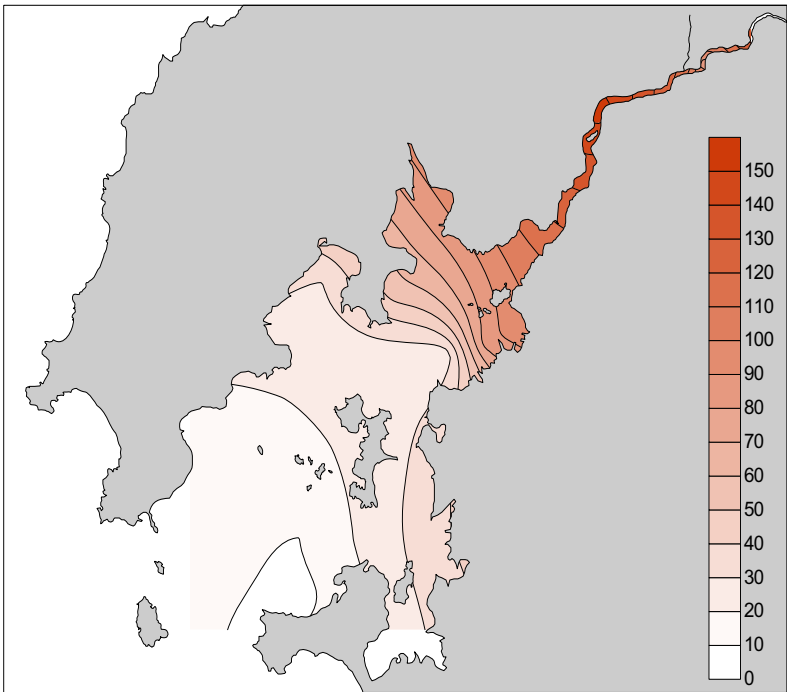


Fig S7b Aluminium isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.

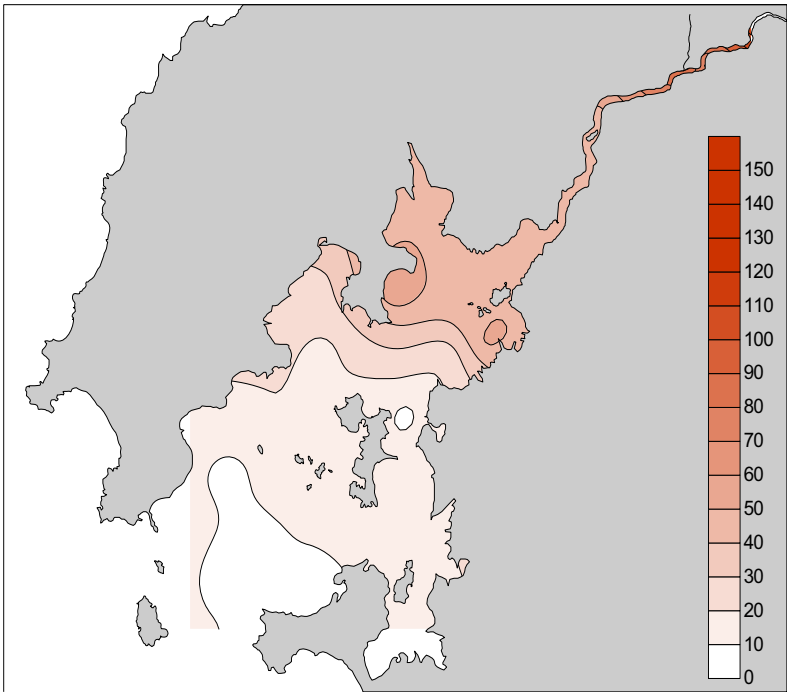


Figure S8a. Zinc isoplot concentration map (μg L^{-1}) for shallow waters at year 2002.

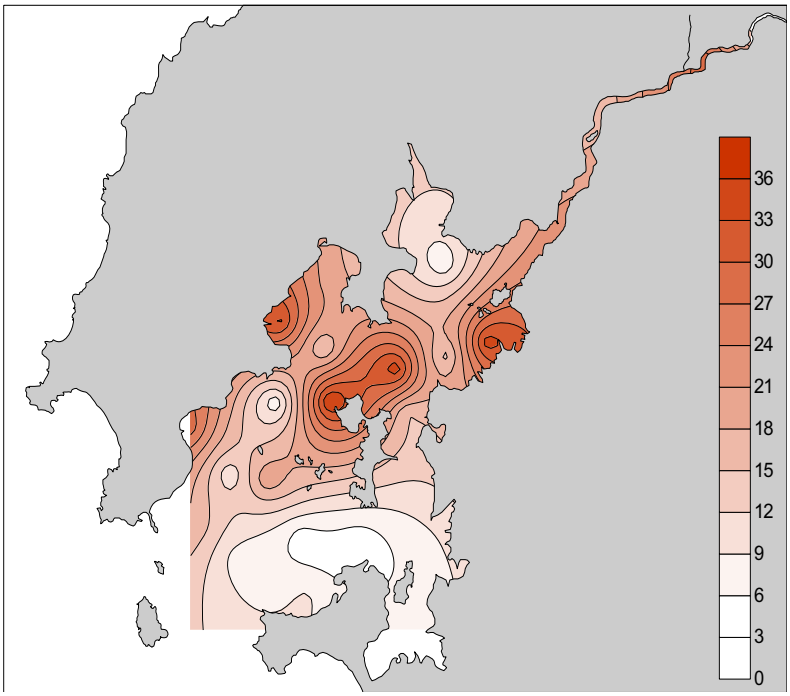
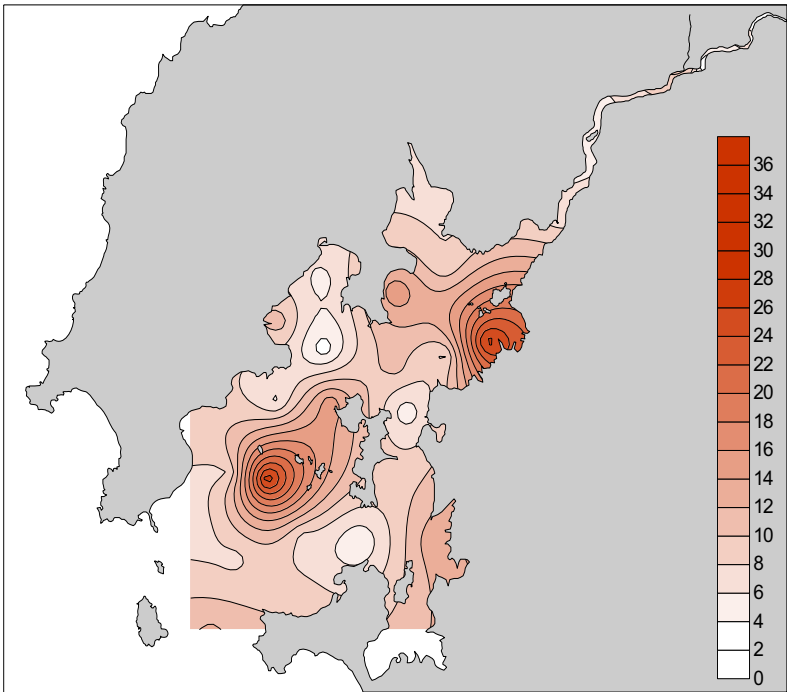


Figure S8b. Zinc isoplot concentration map (μg L^{-1}) for shallow waters at year 2003.



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CONCLUSIONES

CONCLUSIONES

Después de la realización del presente trabajo de investigación se han obtenido las siguientes conclusiones:

1 - Se ha desarrollado un método de extracción en fase sólida para la preconcentración de metales traza en el agua de mar consistente en la complejación de los metales con 8–hidroxiquinolina (8-HQ), seguido de la adsorción de los complejos formados en un cartucho comercial de sílice C18 y la elución de los metales adsorbidos con un pequeño volumen de ácido nítrico 2M para su posterior determinación mediante ICP-OES.
El método propuesto presenta bajos límites de detección y una buena precisión y exactitud, permitiendo la determinación simultanea de Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn y Zn. Sin embargo, resulta necesaria la realización de una recta de adición estándar para determinar dichos elementos, debido a la existencia de un cierto efecto matriz, como consecuencia de las sales disueltas en el agua de mar. Además, aunque los cartuchos de C18 se venden como "de un solo uso", se ha demostrado que pueden ser reutilizados por lo menos siete veces, sin perder sus propiedades adsorbentes.

2 - El método desarrollado ha sido aplicado al estudio de las aguas de la Ría de Arousa, donde se evaluó si el accidente del petrolero "Prestige", en noviembre de 2002, ha tenido alguna influencia sobre la concentración de dichos elementos, obteniéndose como resultado que las concentraciones de las muestras estudiadas se encuentran dentro los niveles de concentración habituales presentes en el resto de Rías gallegas y, por lo tanto, que el accidente del petrolero no ha tenido un efecto significativo en lo que a la contaminación de las aguas por metales pesados se refiere.

3 – Se han sintetizado y caracterizado diversos polímeros de impronta iónica, empleando diferentes plantillas, monómeros y agentes complejantes, siguiendo una novedosa metodología de síntesis polimérica denominada "polimerización por precipitación". De los diferentes materiales preparados, los mejores resultados, en cuanto al reconocimiento del analito plantilla, fueron obtenidos con aquellos polímeros sintetizados en presencia de un agente complejante y del 2-dietilamino etil metacrilato (DEM) como monómero.

4 - La precisión y exactitud de los procedimientos de extracción en fase sólida en los que los polímeros sintetizados ha sido contrastada mediante la realización de diversos estudios de reproducibilidad y recuperación analítica, y mediante el análisis de diferentes materiales de referencia certificados, empleándose distintas técnicas para realizar las determinaciones (ETAAS, ICP-OES, ICP-MS).

5 - Se ha demostrado que los polímeros sintetizados no retienen los elementos mayoritarios presentes en el agua de mar (sodio, potasio, calcio, magnesio), ofreciendo la posibilidad de eliminar eficazmente la matriz salina de las muestras y realizar la determinación directa del analito en los eluatos mediante la realización de una sencilla recta de calibrado. Además, se ha demostrado que los polímeros sintetizados pueden ser reutilizados en varias ocasiones sin perder sus propiedades adsorbentes.

6 - Se ha demostrado que el tamaño y las características del analito plantilla desempeñan un papel muy importante en las propiedades del polímero resultante. También se ha demostrado que la inmovilización química del agente complejante en la matriz polimérica (a través de la inserción de un grupo vinilo polimerizable) confiere

selectividad al polímero y que el reconocimiento del analito plantilla no tiene lugar sin la formación previa de un complejo de prepolimerización en el que el monómero y el agente complejante se puedan organizar adecuadamente alrededor de la plantilla, de modo que, en el polímero resultante, sus grupos funcionales se encuentren dispuestos en la posición geométrica adecuada.

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Evaluation of commercial C18 cartridges for trace elements solid phase extraction from seawater followed by inductively coupled plasma-optical emission spectrometry determination

Jacobo Otero-Romaní, Antonio Moreda-Piñeiro, Adela Bermejo-Barrera, Pilar Bermejo-Barrera*

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avda. das Ciencias, s/n, 15782 Santiago de Compostela, Spain

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Abstract

Possibilities of the use of commercial C18 cartridges to separate and preconcentrate trace elements from seawater have been evaluated. Trace elements (Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn) were previously complexed with 8-hydroxyquinoline, 8-HQ (5×10^{-4} M as final concentration) at alkaline pH (8.0 ± 0.1) and then they were eluted with 2.5 ml of 2.0 M nitric acid. Metals eluted from cartridges were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES). Variables affecting the metal-8-HQ complexation such as pH and 8-HQ concentration, and affecting the metal-8-HQ complexes solid phase adsorption and elution (load and elution flow rates and concentration and volume of eluting solution) were studied in order to find compromise operating conditions for the simultaneous metals complexation, adsorption and elution. After studies of contamination of commercial C18 cartridges for trace metals, high reagent blanks were reached for Ti so that they are useless for the determination of this element. In addition, commercial C18 cartridges can be used at least seven times without loss of adsorption properties. Working with a seawater sample volume of 100 ml and using an optimum nitric acid volume of 2.5 ml for elution, a preconcentration factor of 40 was achieved, factor high enough to determine trace elements in unpolluted surface seawater samples by ICP-OES. Analytical performances, such as limits of detection and quantification, repeatability of the overall procedure and accuracy, by analyzing saline (CASS-3 and SLEW-3) and non-saline (TM-24) certified reference materials, were finally assessed.

Keywords: Commercial C18 cartridges; Solid phase extraction; Trace metals; Unpolluted surface seawater; Inductively coupled plasma-optical emission spectrometry

1. Introduction

Seawater analysis for heavy metals and other contaminants is increasing in contamination studies owing to the need to guarantee the good quality of seawater for different purposes. This is especially important for coastal or estuarine seawater because of the large number of industries dealing with seafood production which use the estuaries to keep, mainly, molluscs and seaweed. In fact, there is European Directive relating to the quality of water to keep molluscs which limits the levels of several heavy metals such as Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn [1].

Since trace metal concentration levels in seawater are very low [2], sensitive analytical techniques as well as preconcentration methods must be used to assess background levels of trace elements. In addition, the saline composition of seawater can be an important drawback in seawater analysis because it is a source of high background signals [3] and, when using inductively coupled plasma-optical emission spectrometry (ICP-OES), a source of spectral [4] and matrix interferences [5]. To overcome these problems, different

* Corresponding author. Tel.: +34 600 942346; fax: +34 981 595012.
E-mail address: pbermejo@usc.es (P. Bermejo-Barrera).

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procedures such as liquid–liquid extraction, coprecipitation, electrodeposition and solid phase extraction, mainly using ionic exchange resins, have been developed. Among all these procedures, solid phase extraction techniques are quite popular since they offer a number of important advantages over other preconcentration methods. One of these benefits is that sorbent material can be packed into mini-columns or cartridges which provide sufficient exchanged/adsorption capacity for many applications [6,7].

The adsorption of non-polar species onto active carbon, silica gel or alumina is well established for non-polar and less polar organic analytes. However, a previous derivatisation stage is mandatory for inorganic species because only uncharged (non-polar) species can interact with the adsorbent material. Therefore, these methods involve a previous complexation of trace metals with an organic chelating reagent [8–12], which must form uncharged metal-complexes. 8-Hydroxyquinoline (8-HQ) forms uncharged chelates with at least 60 elements [13,14] and offers as advantage its lack of affinity for alkaline and alkaline earth metals. Some works dealing with the adsorption of metal-oxinates, such as the applications by Abbasse et al. [7,15] and Skinner and Salin [16], can be found in literature and all these procedures use high purity C18 adsorbent material laboratory-packed into columns.

One of the aims of the current work is evaluating the possibilities of use of commercial C18 cartridges, commonly used for non-polar organic compounds pre-concentration and for clean-up purposes, for trace metals solid phase extraction. This evaluation has implied a study on the trace metals released from new commercial C18 cartridges, and the findings of a procedure to wash and prepare the cartridges for trace elements. In addition, the possibility of reusing the C18 cartridges was also considered in this study.

2. Experimental

2.1. Instrumentation

An Optima 3300 DV inductively coupled plasma-optical emission spectrometer (Perkin-Elmer, Norwalk, USA) equipped with an autosampler AS 91 (Perkin-Elmer) and a GemCone cross flow nebulizer type (Perkin-Elmer) and a cyclonic spray chamber (Perkin-Elmer) was used for all measurements. An 8-way Gilson peristaltic pump (Gilson, Villiers, France) equipped with 3.18 mm i.d. Tygon tubes (Gilson) was used to drive samples through the C18 chemically bonded silica gel (Sep-Pack Plus – 360 mg, Waters, Milford, MA, USA). A Class-100 clean fume hood (Telstar S.A., Terrassa, Spain) was used to perform all experiments in order to avoid contamination of samples and reagents.

2.2. Reagents

Ultra-pure water of resistivity 18 MΩ cm obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). High purity nitric acid and hydrochloric acid were from Panreac (Barcelona, Spain). High purity ammonia and analytical grade methanol were purchased from Merck (Darmstad, Germany). 8-Hydroxyquinoline solution (0.5 M) was prepared from analytical grade reagent (Merck) by dissolving in a 2.0 M hydrochloric acid solution. Ammonium acetate (0.01 M) was prepared from analytical grade reagent ammonium acetate (Merck) by dissolving in Milli-Q water. Multi-elemental standard solution containing As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, Ti and Zn at 100 $\mu g\,ml^{-1}$ was obtained from Perkin-Elmer. Single standard solutions of As, Cd, Cr, Ni, Pb, V, Sn and Zn (Merck), Al, Cu and Fe (Perkin-Elmer) and Mn (Panreac) at 1000 $\mu g\,ml^{-1}$ were also used. Coastal seawater (CASS-3) and estuarine seawater (SLEW-3) certified reference materials were obtained from the National Research Council of Canada. Lake water (TM-24) certified reference material was purchased by the National Water Research Institute of Canada.

All glass- and plastic-ware were rigorously cleaned and kept into 10% (m/m) nitric acid for at least 48 h. The material was then rinsed three times with Milli-Q water.

2.3. Seawater collection

Seawater samples were collected from the Ría de Muros-Noia estuary (north-west Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were acidified at pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption onto the inner bottles walls. Acidified seawater samples were then filtered through a 0.45 μm polycarbonate membrane Nucleopore filter (Millipore).

2.4. C18 cartridge preparation

C18 sorbent packing material was firstly washed by passing 25 ml of 2.0 M nitric acid solution at a flow rate of 1.5 ml min^{-1} in order to remove trace metals adsorbed in the packing material. Then, the C18 cartridges were rinsed by pumping 25 ml of Milli-Q water and they were activated by washing with 10 ml of methanol and rinsed with 10 ml of Milli-Q water. Finally, the activated silica into cartridges was conditioned by passing 10 ml of 0.01 M ammonium acetate (pH 7.0).

2.5. C18 cartridge solid phase extraction procedure

A volume of 0.1 ml of 0.5 M 8-HQ solution was added to 100 ml of acidified seawater samples, giving a final 8-HQ concentration of 5×10^{-4} M. Then, the pH was readjusted to 8.0 ± 0.1 by addition of a small volume (1–2 ml) of a 5.0 M ammonia solution. The seawater sample was then passed through a cleaned and conditioned C18 cartridge at a fixed flow rate of 6.0 ml min^{-1} by using a peristaltic pump. The cartridges were then rinsed with 10 ml of 0.01 M ammonium acetate in order to remove the saline matrix, and the

adsorbed metal-complexes were eluted with 2.5 ml of 2.0 M nitric acid at a flow rate of 4.5 ml min^{-1}. A pre-concentration factor of 40 was achieved under these operating conditions.

2.6. ICP-OES measurements

Eluted trace elements were measured by ICP-OES (axial configuration, radiofrequency power of 1300 W and plasma, auxiliary and nebulizer gas flows of 15.0, 0.5 and 0.8 l min^{-1}, respectively) without dilution. The standard addition method, covering analyte concentrations of 0, 0.04, 0.08 and 0.16 mg l^{-1} (Cd, Cu, Ni, Pb and V); 0, 0.2, 0.4 and 0.8 mg l^{-1} (Mn and Zn); 0, 0.4, 0.8 and 1.6 mg l^{-1} (Sn); 0, 1.0, 2.0 and 4.0 mg l^{-1} (Fe) and 0, 2.0, 4.0 and 8.0 mg l^{-1} (Al), taking into account a pre-concentration factor of 40, was used.

3. Results and discussion

In order to verify possible trace metal contamination of commercial C18 cartridges, five different aliquots (5 ml each one) of 2.0 M nitric acid solution were passed through the cartridges at a flow rate of 1.5 ml min^{-1}. The five sequential eluates, as well as 5 ml of a 2.0 M nitric acid solution (blank), were analysed by ICP-OES. Negligible concentrations in the five washing solutions were found for As, Cd, Cr, Cu, Mn, Ni and Pb. However, contamination was observed for Al, Fe, Ti, Sn and Zn, mainly after passing the first 5 ml nitric acid aliquot (Fig. 1). These metal concentrations were reduced moreover the volume of nitric acid solution was increased and after passing 25 ml of the washing solution (the last 5 ml aliquot) the concentration of most of them were similar than those metal concentrations in 2.0 M nitric acid solution (blank). However, Ti concentrations found in the last washing solution was still extremely high, around 97 μg l^{-1}. Contamination of C18 cartridges by Ti must be probably due to the use of Ti-based devices for grinding and packing the adsorbent material. Further experiments, implying a more concentrated nitric acid solution as well as larger volumes of washing solution, were carried out in order to reduce the levels of Ti before using the cartridges for trace elements preconcentration. All these experiments have resulted in high levels of Ti released from C18 cartridges. Therefore, Ti was not considered in further studies because of high Ti levels released from commercial C18 cartridges.

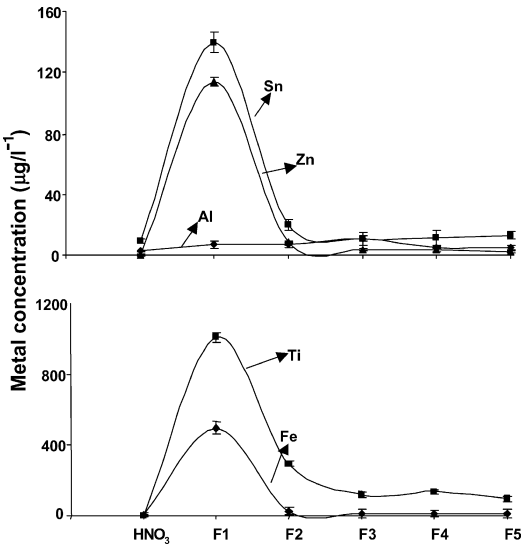

Fig. 1. Concentrations of selected metals in 2.0 M nitric acid washing solutions after passing through commercial Sep-Pack Plus C18 cartridges.

3.1. C18 cartridges solid phase extraction

Preliminary studies on the effect of the 8-HQ concentration on the formation of metal-8-HQ complexes have revealed that this variable is not significant. Therefore, an excess of 8-HQ concentration of 5×10^{-4} M to guarantee the formation of neutral metal-oxinates [14] was used for all experiments. This 8-HQ concentration is quite similar than those previously reported by other authors [7,16].

3.1.1. Effect of the pH on the metal-complexes formation

The efficiency of 8-HQ to react with metals and form uncharged metal-8-HQ complexes is largely dependent on pH. This is because 8-HQ is an ampholyte, forming oxinium (8-hydroxyquinolinium) ion by protonation of N in acid solutions and oxinate ion in basic solutions [13]. Most of the divalent and trivalent elements react with the oxinate form and the establishment of optimum pH values for the different elements is necessary.

To study the effect of pH on the efficiency of metal-8-HQ complexes formation, different aliquots of 100 ml from an acidified seawater sample were spiked with 3 μg l^{-1} of each metal. Trace metals were complexed with 8-HQ and the pH was readjusted by adding small volumes of 5.0 M ammonia solution to obtain pH values within the 7.5–9.5 range. Each pH value was tested three times. Solutions were passed through the pre-conditioned commercial C18 cartridges at a fixed load flow rate of 1.5 ml min^{-1} by using a peristaltic pump. The cartridges were then rinsed with 10 ml of 0.01 M ammonium acetate in order to remove the saline matrix, and the adsorbed metal-8-HQ were eluted with 5.0 ml of 2.0 M nitric acid at an elution flow rate of 0.6 ml min^{-1}. Trace metal concentrations for each experiment at each pH value after analyzing the spiked seawater samples using metal standards in 2.0 M nitric acid for calibration are plotted in Fig. 2.

It can be seen that most of the studied metals are largely formed/retained at pH 8.0. Elements such as Cu, Ni, Fe, Sn and Pb appear not being dependent on the pH value, while As, Cd and Mn are efficiently complexed and retained at pHs within 8.0–9.5 range, decreasing at pHs lower than 8.0.

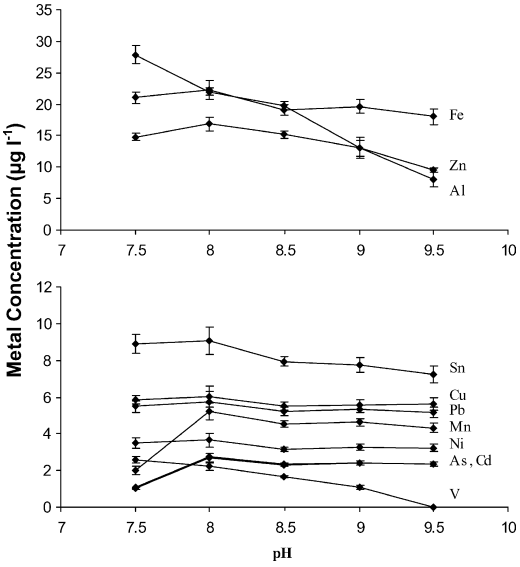

Fig. 2. Effect of the pH on the metal complexation with 8-HQ.

However, V and Al are not complexed and/or retained at high pH values, and a decrease on the formation/adsorption efficiency is observed for pH higher than 7.5. This result can be expected for V, because the most stable species is VO^{2+}, which is only complexed by 8-HQ in acid medium (pH 7.0 or lower than 7). At alkaline pH, VO^{2+} is oxidised to VO_4^{3-}, species which is not complexed by the oxinate form of 8-HQ [7]. Finally, Zn offers a particular trend, showing decreases on the solid phase extraction efficiency when using pH higher and lower than 8.0. Therefore and in order to fix compromise conditions for all elements tested, a pH of 8.0 was chosen, pH that results adequate for most of the elements studied, except for Al and V, for which a pH close to 7 would be the best.

3.1.2. Effect of volume and concentration of nitric acid for elution

Elution conditions were carefully studied. Since uncharged metal-8-HQ complexes are adsorbed onto the adsorbent packing material in C18 cartridges, elution of metals must imply the metal-8-HQ destruction, obtaining divalent and trivalent charged metals which do not interact with the adsorbent. This is easily reached using an acid [7,15] as eluting solution or using methanol [16]. Therefore, different nitric acid solutions were tested in order to elute metals. Two variables affecting metals elution were studied: the nitric acid concentration and the nitric acid volume. This last variable should adopt the lowest value so that a large pre-concentration factor could be assessed. Therefore, two nitric acid solutions at 2.0 and 4.0 M were simultaneously studied for eluting volumes between 2.5 and 6.0 ml (pre-concentration factors within the 40.0–16.7 range). The eluting volume of 2.5 ml was the lowest volume studied because the ICP-OES used requires at least 2.0 ml to perform the measurements. It must be also noticed that there are many systems that can work with smaller volumes, hence higher preconcentration factor may be possible.

Under optimum values for pH, metal-8-HQ retained on the C18 cartridge were eluted at a fixed flow rate of 0.6 ml min^{-1} using different nitric acid volumes and nitric acid concentrations. Results have shown that efficient metals elution are reached under all nitric acid volumes when using 2.0 M nitric acid as eluting solution Similar results have been obtained when using 4.0 M nitric acid. Therefore, the lowest nitric acid concentration (2.0 M) and the lowest nitric acid volume (2.5 ml) were chosen, implying a pre-concentration factor of 40.

3.1.3. Effect of the load flow rate on the metal-complexes solid phase extraction

Load flow rate through the C18 cartridges should be fast enough to perform the solid phase extraction in a short time, but it should be slow enough to allow the interaction between the metal-complexes and the adsorbent packing material in the cartridges.

To study this variable, different aliquots of 100 ml from an acidified seawater sample spiked with 3 µg l^{-1} of each metal were 8-HQ complexed at a pH 8.0 and the solutions were loaded on the C18 cartridges at different flow rates, from 1.5 to 9.0 ml min^{-1}. After sample load, the cartridges were rinsed with 0.01 M ammonium acetate solution (10 ml) and the retained metal-8-HQ were eluted with 2.5 ml of a 2.0 M nitric acid at a flow rate of 0.6 ml min^{-1}. Results have shown that 8-HQ can be retained when pumping solutions at flow rates within the 1.5 and 6.0 ml min^{-1}. Larger load flow rates than 6.0 ml min^{-1} has led to uncompleted adsorption for some elements, such as Mn. Therefore, a sample load flow rate of 6.0 ml min^{-1} was selected. This rate is high enough to load the sample in a moderate short time and so that metal-8-HQ can interact with the adsorbent material.

3.1.4. Effect of the elution flow rate

The effect of elution flow rate was finally studied. After fixing all variables at optimum values (pH 8.0, load flow rate 6.0 ml min^{-1} and 2.5 ml of 2.0 M nitric acid for elution) metal-8-HQ retained were eluted at flow rates from 0.6 to 4.5 ml min^{-1}. Results showed that metals are efficiently eluted from cartridges at all elution flow rates studied and an elution flow rate of 4.5 min^{-1} was selected.

3.1.5. Study of the number of solid phase extraction performed with conventional C18 cartridges

Commercial C18 cartridges, although sold as single use cartridges, were tested in order to know the number of sequential solid phase extraction that can be performed without losses on metal-8-HQ adsorption and/or metals elution. This experiment was carried out with three different cartridges which were used several times for a period of 2 months.

Different seawater samples spiked with variable concentrations of metals were determined and the analytical recoveries for each element obtained. Results have shown that some elements such as Cu, Fe, Pb, Sn and Zn are not efficiently retained/eluted after seven uses, and analytical recovery is poor when using the same C18 cartridges more than seven times. In addition, it has been found that for some elements, even being efficiently retained after seven uses, the repeatability got worse when using the cartridges more than seven times. Therefore, conventional C18 cartridges can be used at least seven times without losing of efficiency of the adsorbent packing material adsorption properties.

3.2. Analytical performance

Although solid phase extraction implies metals separation from the seawater matrix, a comparison between calibration matched with 2.0 M nitric acid and standard addition was established for each element in order to study possible matrix effect. The standard addition technique was obtained after spiking different 100 ml aliquots from the same acidified seawater sample by triplicate with different metal concentrations (up to 4.0 $\mu g\,l^{-1}$ for As, Cd, Cu, Ni, Pb and V, 20 $\mu g\,l^{-1}$ for Mn and Zn, 40 $\mu g\,l^{-1}$ for Sn, 100 $\mu g\,l^{-1}$ for Fe and 200 $\mu g\,l^{-1}$ for Al). After solid phase extraction, the eluates were measured by ICP-OES (Section 2.6) and 2.0 M nitric acid calibrations and standard addition graphs were obtained. The standard deviation and the mean for the slopes of two standard addition graphs and six external 2.0 M nitric acid calibrations were statistically compared by using the Cochran'C and Bartlett's tests at 95.0% to compare variances, and the ANOVA test to compare means. It can be said that slopes for external 2.0 M nitric acid calibration and standard addition graphs are statistically different for all cases, except for V. Therefore, although a large volume of ammonium acetate solution was used to remove possible salts on C18 cartridges just before metals elution, a certain matrix effect was observed when determining all elements, being necessary the establishment of a standard addition graph to perform seawater analysis.

The limit of detection, given by LOD = 3(S.D./*m*), where S.D. is the standard deviation of eleven measurements of a reagent blank, and *m* the slope of the standard addition graph, were calculated. After a pre-concentration factor of 40, LODs of 1.28, 0.04, 0.04, 0.02, 0.19, 0.001, 0.03, 0.03, 0.07, 0.06 and 1.13 $\mu g\,l^{-1}$, were reached for Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn, respectively. Such LODs are low enough to determine trace metals levels in unpolluted seawater samples.

The repeatability of the over-all procedure (solid phase extraction and ICP-OES determination) was assessed by analyzing three times a same seawater sample. The average percent relative standard deviations (R.S.D.s) were 3.5, 0.7, 3.7, 6.3, 4.7, 5.3, 0.2, 5.2, 5.4, 7.5 and 3.5% for Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn, respectively. Since these results it can be concluded that the procedure offer good repeatability for all elements (R.S.D.s lower than 10%).

Table 1
Percent analytical recoveries ($n = 6$) of the method

	Percent analytical recoveries		
	Low[a]	Medium[b]	High[c]
Al	87.3 ± 3.0	92.0 ± 1.3	101.2 ± 1.7
As	102.3 ± 1.5	101.6 ± 0.2	100.1 ± 0.1
Cd	103.3 ± 0.8	101.7 ± 0.4	100.3 ± 0.1
Cu	107.6 ± 1.7	101.9 ± 3.2	100.9 ± 0.5
Fe	95.1 ± 2.3	98.7 ± 1.9	99.7 ± 0.3
Mn	104.9 ± 4.0	102.1 ± 0.9	100.3 ± 0.3
Ni	106.3 ± 1.3	104.7 ± 2.7	100.8 ± 0.8
Pb	98.3 ± 1.8	99.0 ± 5.2	99.9 ± 0.1
Sn	92.2 ± 0.3	92.5 ± 5.3	99.8 ± 0.3
V	70.7 ± 5.2	97.9 ± 0.6	87.7 ± 6.6
Zn	108.2 ± 0.4	102.7 ± 3.4	100.2 ± 0.7

[a] 1 $\mu g\,l^{-1}$ for As, Cd, Cu, Ni, Pb and V; 5 $\mu g\,l^{-1}$ for Mn and Zn; 10 $\mu g\,l^{-1}$ for Sn; 25 $\mu g\,l^{-1}$ for Fe and 50 $\mu g\,l^{-1}$ for Al.
[b] 2 $\mu g\,l^{-1}$ for As, Cd, Cu, Ni, Pb and V; 10 $\mu g\,l^{-1}$ for Mn and Zn; 20 $\mu g\,l^{-1}$ for Sn; 50 $\mu g\,l^{-1}$ for Fe and 100 $\mu g\,l^{-1}$ for Al.
[c] 4 $\mu g\,l^{-1}$ for As, Cd, Cu, Ni, Pb and V; 20 $\mu g\,l^{-1}$ for Mn and Zn; 40 $\mu g\,l^{-1}$ for Sn; 100 $\mu g\,l^{-1}$ for Fe and 200 $\mu g\,l^{-1}$ for Al.

The accuracy of the method was verified by studying the analytical recovery and by analyzing different saline water certified reference materials (CASS-3 and SLEW-3) and a non-saline water certified reference material (TM-24). Analytical recovery was assessed for three concentration levels, after spiking two different seawater samples with analyte concentrations of 1.0, 2.0 and 4.0 $\mu g\,l^{-1}$ for As, Cd, Cu, Ni, Pb and V; 5, 10 and 20 $\mu g\,l^{-1}$ for Mn and Zn; 10, 20 and 40 $\mu g\,l^{-1}$ for Sn; 25, 50 and 100 $\mu g\,l^{-1}$ for Fe and 50, 100 and 200 $\mu g\,l^{-1}$ for Al. The concentration levels tried are representative of clean surface seawaters. Each concentration level in each seawater samples was tried three times, thus, analytical recoveries listed in Table 1 are the mean of six independent measurements. From Table 1 it can be concluded that good analytical recovery (within the 90–110% range) are reached for all elements at all concentration levels, except for V at the lowest concentration level. This could be attributed to a less efficient solid phase extraction for V for which a more acidic pH (lower than 8.0) would be desirable.

CASS-3, SLEW-3 and TM-24 certified reference materials were analyzed by triplicate for most of the elements. Determinations were performed by using the standard addition technique. Results, listed in Table 2, reveal good agreement between found concentrations and certified values for most of the elements in the three certified reference materials. However, Cd, Pb and Zn have not been determined in CASS-3 and SLEW-3 because certified concentrations of these elements in these materials are lower than the LODs of the method for these elements. In addition, V concentration found is not within the V certified concentration range due to a pH of 8.0 is not the optimum to form V-8-HQ complexes (see Fig. 2). Finally and although As levels in the three certified reference materials are quite higher than the LOD of the method for this element, As in the three certified reference materials was not determined. Arsenic is present mainly as $HAsO_4^{2-}$ (As(V))

Table 2
Analysis of certified reference materials ($n = 3$)

	SLEW-3		CASS-3		TM-24	
	Certified ($\mu g\,l^{-1}$)	Found ($\mu g\,l^{-1}$)	Certified ($\mu g\,l^{-1}$)	Found ($\mu g\,l^{-1}$)	Certified ($\mu g\,l^{-1}$)	Found ($\mu g\,l^{-1}$)
Al	–	–	–	–	22 ± 19	28 ± 1
As	1.36 ± 0.09	[a]	1.09 ± 0.07	[a]	2.7 ± 1.0	[a]
Cd	0.048 ± 0.004	<LOD	0.030 ± 0.005	<LOD	12.5 ± 3.5	14.6 ± 1.0
Cu	1.55 ± 0.12	1.96 ± 0.12	0.517 ± 0.062	0.648 ± 0.060	8.0 ± 4.1	9.0 ± 0.3
Fe	0.568 ± 0.059	0.520 ± 0.002	1.26 ± 0.17	1.09 ± 0.12	5.4 ± 2.3	5.4 ± 0.5
Mn	1.61 ± 0.22	1.62 ± 0.19	2.51 ± 0.36	2.78 ± 0.38	3.1 ± 1.4	2.9 ± 0.1
Ni	1.23 ± 0.07	1.35 ± 0.13	0.386 ± 0.062	0.353 ± 0.033	3.5 ± 3.0	3.7 ± 0.1
Pb	0.0090 ± 0.0014	<LOD	0.012 ± 0.004	<LOD	7.3 ± 2.6	9.7 ± 0.2
V	2.57 ± 0.31	1.96 ± 0.16	–	–	6.7 ± 2.0	4.0 ± 1.0
Zn	0.201 ± 0.037	<LOD	1.24 ± 0.25	<LOD	13.6 ± 4.8	20.8 ± 0.4

[a] Not determined.

in seawater [2] but 8-HQ only reacts with As(III) species. Therefore, good analytical recovery is reached when spiking with As(III), as shown in Table 1, but lack of accuracy has been obtained after analyzing certified reference materials, in which As occurs as As(V).

4. Conclusions

The use of commercial Sep-Pack Plus C18 silica cartridges, commonly used for non-polar organic analytes, can be used to pre-concentrate trace elements from seawater after 8-HQ complexation. A previous washing step, involving the use of 25 ml of 2.0 M nitric acid, is required to avoid contamination from the silica adsorbent packing material. After this wash treatment, contamination problems are significant for Ti and this element cannot be pre-concentrated because of high reagent blanks. In addition, although C18 cartridges are sold as single use cartridges, they can be reused at least seven times without losing of analytical performances. Compromise conditions for different variables affecting the metal-8-HQ formation and the adsorption on and elution from the adsorbent silica allow the simultaneous solid phase extraction of Al, Cd, Cu, Fe, Mn, Ni, Pb, Sn, V and Zn from seawater. The optimized solid phase extraction is fast and it offers a high sample treatment rate (simultaneous solid phase extraction of eight samples can be carried out using an 8-way peristaltic pump in 30 min). In addition, the sample volume required is low when comparing to other proposed methods [7,16], which imply a fast sampling and advantages when storing samples. This fact combined with the adequate sensitivity by ICP-OES of new generation allows the application of this method to routine multi-element analysis of seawater.

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Synthesis, characterization and evaluation of ionic-imprinted polymers for solid-phase extraction of nickel from seawater

Jacobo Otero-Romaní[a], *Antonio Moreda-Piñeiro*[a], *Pilar Bermejo-Barrera*[a,*], *Antonio Martin-Esteban*[b]

[a] *Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n. 15782 – Santiago de Compostela, Spain*
[b] *Department of Environment, National Institute for Agriculture and Food Research (INIA), Carretera de A Coruña, Km 7,5, 28040 Madrid, Spain*

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ABSTRACT

Several nickel ion imprinted polymers were prepared via precipitation polymerization using 4-vinylpyridine or 2-(diethylamino) ethyl methacrylate as monomers (vinylated reagents) and a cross-linking agent divinylbenzene in the presence of nickel(II) alone or nickel(II) and 8-hydroxyquinoline (non-vinylated reagent). For all cases, 2,2′-azobisisobutyronitrile (AIBN) was used as an initiator and an acetonitrile/toluene (3:1) mixture was chosen as a porogen. After packing the polymer particles into empty SPE cartridges, nickel(II) ions were removed by washing with 50 mL of 2.0 M nitric acid. Characterization of the polymer particles has been carried out by scanning electron microscopy, energy dispersive X-ray fluorescence and elemental analysis. The best nickel imprinting properties were given by polymers synthesized in the presence of 8-hydroxyquinoline and 2-(diethylamino) ethyl methacrylate as a monomer. The optimum pH for quantitative nickel retention was 8.5 ± 0.5, while elution was completed with 2.5 mL of 2.0 M nitric acid. When using polymer masses of 300 mg, sample volumes until 250 mL can be passed through the cartridges without reaching the breakthrough volume. Therefore, a pre-concentration factor of 100 has been reached when eluting with 2.5 mL of the elution solution. Electrothermal atomic absorption spectrometry has been used as a detector for nickel determination. The limit of detection of the method was $0.050\,\mu g\,L^{-1}$ (pre-concentration factor of 100), while the relative standard deviation for eleven replicates was 6%. Accuracy of the method was assessed by analyzing different certified reference materials: SLEW-3 (estuarine water) and TM-23.3 and TM-24 (lake water).

1. Introduction

Electrothermal atomic absorption spectrometry (ETAAS) and multi-element detectors such as inductively coupled plasma—optical emission spectrometry/mass spectrometry (ICP-OES/MS) have been commonly used to assess trace elements in waters. However, lack of sensitivity and accuracy can be found when using these techniques for seawater analysis.

* *Corresponding author*. Tel.: +34 981 563100x14266; fax: +34 981 595012.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).
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As recently reviewed by Rao et al. [1] ETAAS combining the multiple injection technique and the Zeeman correction has been used for direct determination of Ni in seawater [2,3]. Although charring temperatures till 1700 °C can be used without nickel losses [2], the seawater matrix (mainly NaCl) has been reported to be held in the graphite tube even at temperatures of 1700 °C [4]. This fact generates high background signals, scattering, and a strong matrix effect in direct ETAAS determinations that lead to a worsening on sensitivity and even loss of accuracy [3]. In addition, spectral and matrix interferences have been also reported for ICP-OES measurements [5,6], and the removal of the salt matrix prior seawater analysis is highly recommended [7].

Therefore, separation/pre-concentration methods are commonly used as previous stages for trace element determinations in seawater samples. Solid-phase extraction (SPE), mainly using ionic exchange resins [8], functionalized chelating resins [9] or high purity C_{18} adsorbent material [7,10,11] are mostly used to pre-concentrate trace elements and to remove the salt matrix. SPE has become quite popular since it offers many practical and operating advantages over other pre-concentration methods [8]. However, interfering compounds might be coextracted with the target analytes on conventional sorbents. To overcome this lack of selectivity, the use of molecularly imprinted polymers (MIPs) have been proposed, especially suited for the clean-up of organic compounds [12]. The synthesis of MIPs is fast and quite cheap and the material provides a high degree of molecular recognition. As reported, molecular imprinting polymerization is based on the preparation of a highly cross-linked polymer around a template in the presence of a suitable monomer [12]. At present, the imprinting of organic molecules is a well-established technology; however, few attempts have been made for ions such as trace elements [13,14]. In such cases, the synthesized sorbent is called an ionic-imprinted polymer (IIP). IIPs have been mostly synthesized for recognizing lanthanides [15–19], actinides [20,21] and noble metals [22]. Recently, a variety of IIPs have been prepared as selective sorbents for the SPE of heavy metals and transition elements such as copper [23,24], cadmium [25,26], cobalt [27], selenium [28] and nickel [27,29,30].

Most of the IIPs have been obtained by bulk polymerization, and the material has to be ground and sieved to obtained particles of an appropriate size range for subsequent use. This process is tedious and time-consuming, and the particles obtained are irregular in size and shape, as well as part of the material is lost as fine dust. In addition, only 50% or less of the total amount of polymer is useful for analytical purposes, and some binding sites are partially destroyed during grinding which leads to a considerable loss of loading capacity of the imprinted polymer [12]. To overcome these problems, different approaches to synthesizing MIPs and IIPs have been proposed by several authors [31,32]. In accordance with Arshady [33], four different polymerization approaches (suspension, emulsion, dispersion and precipitation methods) can be carried out. In the first one, the initiator is soluble in the monomer, and these two are insoluble in the porogen. Therefore, there are two phases in which the monomer phase is suspended in the porogen by means of a stirrer and a suitable droplet stabilizer (suspension agent). Polymerization leads to the monomer "microdroplets" are converted directly to the corresponding polymer "microbeads" of approximately the same size. Emulsion polymerization uses a monomer insoluble in the porogen while the initiator is soluble in the polymerization medium and not in the monomer. This approach requires a surfactant in order to emulsify the two phases (monomer and porogen). Under these conditions, the monomer is present in the mixture partly in the form of droplets and partly in the form of micelles and a small percentage of it can also be molecularly dissolved in the porogen. Since the initiator is in the porogen, polymerization starts in the porogen and the formed oligoradicals are either surrounded by the dissolved monomer and the emulsifier molecules, or they are absorbed by the micelles. These emulsifier-stabilized structures gradually grow until the monomer is consumed. In either dispersion or precipitation polymerization methods the monomer and the initiator are both soluble in the porogen and the polymerization is initiated in homogenous solution and in the presence of a larger amount of porogen [34]. The difference between both methods is mainly attributed to the swollen capacity of the first primary particles. In the dispersion method, the particles are swollen by the porogen and/or the monomer while in the precipitation method, the first particles do not swell in the solvent. For both methods, the polymer particles form a different phase (solid phase) depending on the solvency of the resulting macromolecules (polymer particles) in the porogen. These techniques have emerged as attractive, simple and seemingly general methods for producing high-quality imprinted products, because crushing and sieving steps are avoided and higher yields of reaction are obtained. Application of dispersion polymerization to ionic imprinting can be found in the literature [22,23,25].

The aim of the current work has been the application of the precipitation polymerization approach to synthesizing different IIPs for nickel retention against major elements in seawater (mainly sodium and potassium). Different precipitation polymerization processes, involving only the template (Ni(II)) and the selected monomer (4-vinylpyridine, 4-VP, or 2-(diethylamino) ethyl methacrylate, DEM) or the vinylated monomer (4-VP or DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ), were carried out. For all cases, the synthesized polymer particles were packed into empty SPE cartridges, and they were evaluated/applied for nickel pre-concentration from seawater samples. Both ETAAS and ICP-OES have been used as selective detectors for nickel determination.

2. Experimental

2.1. *Apparatus*

A PerkinElmer Model 1100B (PerkinElmer, Norwalk, CT, USA) atomic absorption spectrometer equipped with an HGA-700 graphite furnace atomizer, deuterium background correction, an AS-70 auto-sampler and a nickel hollow cathode lamp (Cathodeon, Cambridge, UK) was used for the determination of nickel. An Optima 3300 DV inductively coupled plasma (ICP) atomic emission spectrometer (PerkinElmer) equipped with an autosampler AS 91 (PerkinElmer) and a Gem-Cone

cross-flow nebulizer type (PerkinElmer) was used for multi-elemental determinations. A temperature-controlled incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. A vacuum manifold station (Waters, Milford, MA, USA) connected to a vacuum pump (Millipore Co., Bedford, MA, USA) was used for SPE. IIPs were packed into 5 mL SPE cartridges (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA). ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Ultra-pure water of resistivity 18 MΩ cm obtained from a Milli-Q purification device (Millipore Co.) was used to prepare all the solutions. High purity nitric acid and analytical grade $NiCl_2{\cdot}6H_2O$ were purchased from Panreac (Barcelona, Spain). Single standard solutions (1000 mg L^{-1}) of Ca, K, Mg, Na and Ni were from Merck (Darmstadt, Germany). High purity ammonia, ammonium chloride, and analytical grade 8-hydroxyquinoline were purchased from Merck. HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). 4-Vinylpyridine (4-VP) and 2-(diethylamino) ethyl methacrylate (DEM) used as monomers were from Sigma–Aldrich (Steinheim, Switzerland). Divinylbenzene-80 (DVB) was from Sigma–Aldrich and was treated in order to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing around 0.5 g of neutral alumina (Sigma–Aldrich). 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at −20 °C after dissolving the reagent in methanol (Merck) at 50–60 °C. After purification, this reagent was stored at 4 °C. Estuarine seawater (SLEW-3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM-24 and TM-23.3) certified reference materials were purchased by the National Water Research Institute of Canada. All glass and plastic material were rigorously cleaned and kept into 10% (m m^{-1}) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before being used.

2.3. Seawater collection

Seawater samples were collected from the Ría de Muros-Noia estuary (north west Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were acidified at a pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then filtered through 0.45 μm polycarbonate membrane Nucleopore filters (Millipore) and stored at low temperature until used.

2.4. ETAAS measurements

Since Ni(II) was eluted from IIPs with a 2.0 M nitric acid solution, several experiments were carried out to determine the optimum temperatures and times for the charring and atomization steps using aqueous Ni(II) solutions (20 μg L^{-1}) in 2.0 M nitric acid. Since nickel is not a volatile element and as the sample solutions are free of salt, chemical modification was not considered. The optimized graphite furnace temperature program consisted of a drying step at 110 °C (ramp rate and hold times of 10 and 20 s, respectively), followed by a charring stage at 1400 °C (ramp rate and hold times of 30 and 10 s, respectively). Atomization was carried out at 2500 °C (maximum power and 5 s as atomization/integration time). All experiments were carried out using pyrolytic coated graphite tubes with L'vov platforms and injection volumes of 20 μL. Negligible background signals were recorded under these optimized operating conditions. For all nickel determinations, aqueous calibration in 2.0 M nitric acid was performed covering nickel concentrations until 50 μg L^{-1}.

2.5. ICP-OES measurements

Nickel and major elements such as sodium, potassium, calcium and magnesium were measured by ICP-OES (axial configuration) using a radiofrequency power of 1300 W and plasma, auxiliary and nebulizer argon flows of 15, 0.5 and 0.8 L min^{-1}. The detection wavelengths were 231.605 nm for Ni and 589.592, 766.490, 285.213 and 315.887 nm for Na, K, Mg and Ca, respectively. Determinations were performed by using aqueous standards in 2.0 M nitric acid. The calibration has covered Ni concentrations within the 0–4 mg L^{-1} range and up to 500 mg L^{-1} for Na, K, Ca and Mg.

2.6. Synthesis of nickel ionic-imprinted polymer particles

Table 1 lists the monomers (vinylated reagents), non-vinylated ligand, cross-linker and free radical initiator as well as the amounts used in each synthesis. IIPs coded as IIP-1 and IIP-2 were synthesized in the absence of 8-HQ, DVB as a cross-linker and AIBN as an initiator. Solid $NiCl_2{\cdot}6H_2O$ (~35 mg) was mixed with 4-VP (71.5 μL) or DEM (120 μL) into 15 mL glass test tubes. *Then*, 12.5 mL porogen (3:1 acetonitrile:toluene) was added and the mixture was stirred for 5 min, and then filtered. Finally, adequate volumes/amounts of DVB and AIBN (Table 1) were added, the glass tubes were purged with N_2 for 10 min at 0 °C (tubes in an ice bath), were immediately sealed, and placed into a temperature-controllable incubator camera equipped with a low-profile roller. The roller allows the slow rotation of the tubes (33 rpm) about its long axis over the course of the polymerization. The temperature was ramped from room temperature to 60 °C over 2 h, and then maintained at 60 °C for a further 24 h.

IIPs coded as IIP-3 to IIP-6 were synthesized in the presence of a non-vinylated ligand (8-HQ). 8-HQ forms uncharged chelates with at least 60 elements [35,36] and offers as an advantage its lack of affinity for alkaline and alkaline earth metals. In this case, the template (~35 mg of $NiCl_2{\cdot}6H_2O$) was mixed with 8-HQ (~45 mg for IIP-3 and IIP-4 or ~85 mg for IIP-5 and IIP-6) and the appropriate volume of monomer (4-VP or DEM) according to Table 3 and 12.5 mL of porogen. After stirring for 5 min the mixture was filtered, the cross-linker and initiator were added (Table 1) and the precipitation polymerization was carried out as described above. It must be noted that IIP-5 and IIP-6 were synthesized in the presence of a

Table 1 – Molar Ni(II)/monomer/ligand ratio and masses and volume of the different reagents involved into the polymerization process.

	Template ($NiCl_2 \cdot 6H_2O$) (mg)	Monomer (4-VP/DEM) (μL)	Ligand (8-HQ) (mg)	Cross-linker (DVB) (μL)	Initiator (AIBN) (mg)	Molar ratio (Ni/monomer/ligand)	Mass of polymer[b] (g)
IIP-1: Ni/4-VP	39.9	71.5		590	46.6	1/2/0	0.3121 (62%)
IIP-2: Ni/DEM	35.5	120		530	42.2	1/2/0	0.3808 (76%)
IIP-3: Ni/4-VP/8-HQ	38.3	34.5	46.7	575	45.46	1/2/2	0.3935 (79%)
IIP-4: Ni/DEM/8-HQ	36.4	61.5	44.5	545	43.2	1/2/2	0.4471 (89%)
IIP-5: Ni/4-VP/8-HQ[a]	35.0	31.5	85.5	525	43.3	1/2/4	0.3412 (68%)
IIP-6: Ni/DEM/8-HQ[a]	33.4	56.5	81.7	500	41.3	1/2/4	0.3000 (60%)

[a] Double mass of 8-HQ.
[b] Efficiency of the polymerization process (theoretical amount of 0.5 g) in brackets.

double amount of 8-HQ respect to IIP-3 and IIP-4. The ratio among the template (Ni(II)), monomer and ligand for each IIP is listed in Table 1. This table also gives the mass of polymer obtained and the efficiency of the polymerization process taking into account a theoretical amount of synthesized polymer of 0.5 g. It can be seen that efficiencies higher than 60% have been achieved for all cases.

The different IIPs were vacuum filtered, washed with acetonitrile, and then oven-dried overnight at 40 °C. Finally, the polymers (100 or 300 mg) were packed into 5 mL cartridges between Teflon frits.

Blank polymer particles (non-imprinted polymers, NIPs) were also prepared in the same way as IIPs, using the molar ratios listed in Table 1 but without the template. The NIPs were then subjected to the same washing pre-treatment as described in the following section.

2.7. *Template removal procedure*

Once the imprinted polymer was prepared, the template (Ni(II) ions) must be removed from the polymer particles, leaving free cavities complementary in size, shape, and functionality ready for analyte recognition. Typically, template can be quantitatively removed from the polymeric matrix by several hours of stirring the polymeric material with 1:1 hydrochloric acid [13] or other reagents such as acidic thiourea [25] or methanol/water and ethylenediaminotetracetic acid (EDTA) [23]. In the current work, the Ni(II) removal from the synthesized materials was carried out by extensively washing with 2.0 M nitric acid (5.0 mL aliquots) once the polymers were packed as SPE cartridges. Negligible nickel concentrations were found in the washing/filtrate solutions after passing 50 mL of 2.0 M nitric acid.

2.8. *IIPs solid-phase extraction*

Nickel aqueous standard solutions were prepared in 100 mL of 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at the convenient pH (optimum value of 8.5). Similarly, 100 mL of acidified seawater sample was treated with 1–2 mL of a 5.0 M ammonia solution, readjusting the pH to 8.5 ± 0.5. The use of ammonia or NH_4Cl/NH_3 buffer solutions is necessary in order to fix the pH and to prevent the transition metal hydroxides precipitation at high pHs. Then, the solutions were passed through cleaned and conditioned IIP cartridges at a fixed flow rate of 10 mL min^{-1} by using a vacuum manifold station. The cartridges were then rinsed with 2.5 mL of the NH_4Cl/NH_3 buffer solution at the same pH *as* that used for the loading solution, and then, the retained nickel ions were subsequently eluted with two 1.25 mL aliquots of 2.0 M nitric acid solution at a flow rate of 1.5 mL min^{-1}. A pre-concentration factor of 40 was achieved under these operating conditions. After elution, the IIPs were treated with 10 mL of Milli-Q water and then conditioned by passing 10 mL of the 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at the working pH (8.5).

3. Results and discussion

3.1. *Preliminary evaluation of the synthesized polymers: effect of pH*

A preliminary evaluation about imprinting properties of the synthesized polymers has been carried out by loading 600 ng of Ni(II) as 3.0 mL aliquots of 200 μg L^{-1} of Ni(II). These solutions were prepared in 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at different pHs ranging from 4 to 9. After loading, the polymers were then rinsed with 3.0 mL of NH_4Cl/NH_3 buffer solution at a fixed pH (the same as the loading solution), and the retained nickel(II) ions were eluted with 3.0 mL of 2.0 M nitric acid. All experiments were carried out by triplicate and all solutions were analyzed by ICP-OES against an aqueous calibration in 2.0 M nitric acid. The effect of the pH on the nickel(II) retention was studied for all IIPs synthesized as well as all NIPs. Results as analytical recoveries are plotted in Fig. 1A for those IIPs synthesized with 4-VP as a monomer and Fig. 1B for IIPs based on the use of DEM as a monomer.

It can be seen that IIP-4 and IIP-6 synthesized with DEM as a monomer and in the presence of 8-HQ gives good recoveries for nickel when working at high pHs (8–9). NIP-4 and NIP-6 have not shown affinity for nickel at any pH, which indicates that specific (imprinting) cavities were produced during the synthesis of IIP-4 and IIP-6. It must be noticed that IIP-2, synthesized with DEM but in the absence of 8-HQ has not shown imprinting properties for Ni. This result agrees with those reported by Metilda et al. for uranium pre-concentration, which showed that uranyl retention is only quantitative when using a IIP based on a ternary complex among uranyl ions, 5,7-dichloroquinoline-8-ol (DCQ) as a non-vinylated agent and

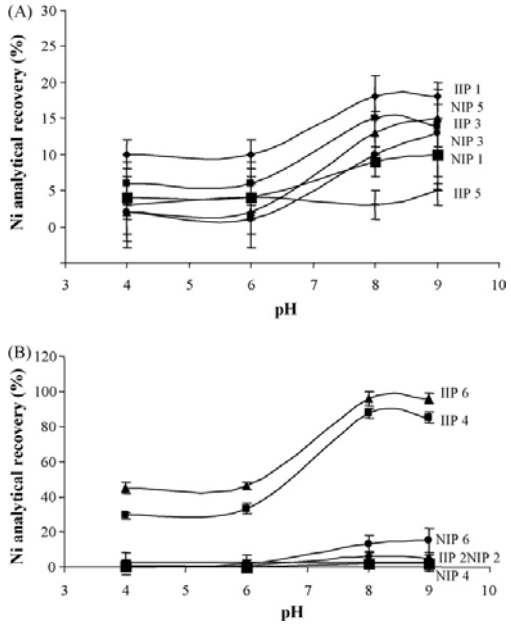

Fig. 1 – Effect of the pH on the nickel analytical recovery for (A) 4-VP based IIPs: IIP-1 (Ni/4-VP, 1/2), IIP-3 (Ni/4-VP/8-HQ, 1/2/2), IIP-5 (Ni/4-VP/8-HQ, 1/2/4), NIP-1 (4-VP), NIP-3 (4-VP/8-HQ, 2/2), NIP-5 (4-VP/8-HQ, 2/4); and for (B) DEM based IIPs: IIP-2 (Ni/DEM, 1/2), IIP-4 (Ni/DEM/8-HQ, 1/2/2), IIP-6 (Ni/DEM/8-HQ, 1/2/4), NIP-2 (DEM), NIP-4 (DEM/8-HQ, 2/2), NIP-6 (DEM/8-HQ, 2/4).

4-VP [37]. The synthesis of IIPs in the presence of DEM and 8-HQ leads to polymerization in the chemical bonding of the monomer, while the non-vinylated ligand is trapped inside the polymeric matrix. The proposed schematic representation of IIP-4 and IIP-6 synthesis is given in Fig. 2. Firstly, the template (Ni(II)) reacts with the ligand to form a nickel(II)-8-HQ binary complex and/or with the monomer and the ligand to form a nickel(II)-8-HQ-DEM ternary complex, which polymerize in the presence of DVB and AIBN through the vinyl groups of DEM [14]. In any case, the ligand (8-HQ) is trapped into the polymeric matrix and offers imprinting cavities for nickel(II). As recently reported, the non-vinylated agent is kept intact in the polymer matrix while leaching the template [13]. This fact have been observed for non-vinylated agents such as DCQ [37], dimethylglioxime and amino-, hydroxyl- or mercapto-quinolines [22] in the cross-linked polymers employing uranyl ions [37] and noble metals [22] as templates.

The trapped 8-HQ plays an important role in nickel(II) recognition because the optimum pH for loading (within 8–9) agrees with reported pHs for nickel-8-HQ complex formation in water [7]. The efficiency of 8-HQ to react with metal ions and form uncharged metal-8-HQ complexes is largely dependent on pH [35]. This is because 8-HQ is an ampholyte, forming oximium (8-hydroxyquinolinium) ion by protonation of N in acid solutions and oxinate ion in basic solutions. 8-HQ is only capable to interact with metal ions in basic solutions, because the hydroxyl group is not protonated [35].

Finally, IIP-1, IIP-3 and IIP-5, all synthesized in the presence of 4-VP as a monomer, have not shown imprinting properties for nickel (analytical recoveries lower than 20% and close similar to those achieved by using the corresponding NIPs).

3.2. *Characterization studies*

3.2.1. *Scanning electron microscopy*

In order to study the morphology and the size of the materials synthesized, scanning electron microscopy (SEM) pictures were taken from IIP-6 and NIP-6 (Fig. 3). It can be seen that spherical monodisperse particles of around 10 μm of diameter were obtained for NIP-6, whereas IIP-6 consisted of agglomerates of different sizes formed by smaller particles. The different polymer morphology can only be attributed to the presence of the template, as previously reported in some works dealing with precipitation polymerization [38], and thus the existence of nickel(II)-8-HQ-DEM complexes leading to the formation of binding sites.

3.2.2. *Energy dispersive X-ray fluorescence studies*

EDXRF patterns for IIP-6 material before and after leaching, as well as for the corresponding NIP-6 were obtained. It has been obtained that nickel was only present in the unleached polymer particles, and it was totally removed after leaching with 50 mL of 2.0 M nitric acid. It was also observed that the spectra of leached IIP-6 and NIP-6 were quite similar.

3.2.3. *Microanalysis studies*

The elemental (H, C, N and O) composition of IIP-6 and NIP-6 were measured and values of 8.5% were found for hydrogen in both IIP-6 and NIP-6, while carbon was 85.6% and 88.2%, for IIP-6 and NIP-6, respectively. Nitrogen percentage was 3.5% and 3.1% for IIP-6 and NIP-6, respectively, and oxygen percentages were 3.3% and 2.8% for IIP-6 and NIP-6, respectively. The good agreement between calculated and experimentally found values of H, C, N and O can indicate that 8-HQ is indeed embedded in the polymeric matrix.

3.3. *Optimization of nickel IIP-SPE from seawater*

3.3.1. *Effect of nitric acid volume/concentration for elution*

Since Ni(II) ions are retained by binding to 8-HQ residues, elution of Ni(II) ions must imply the metal-8-HQ bonding destruction. This is easily reached by using acidic conditions. A first set of experiments were carried out in order to find the optimum nitric acid concentration for the eluting solution. 25 mL aliquots of Ni(II) aqueous standard solutions of 50 $\mu g\,L^{-1}$ were prepared in 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at pH 8.5 and were passed through the cartridges loaded with 100 mg of IIP-6 under operating conditions shown in Section 2.8. After rinsing, the retained analyte was eluted by passing volumes of 3.0 mL of nitric acid at concentrations between 2.0 and 5.0 M and these solutions were measured by ICP-OES against an aqueous calibration in 2.0 M nitric acid. The same analytical recoveries were obtained for all tested nitric acid concentrations, so an eluting solution of 2.0 M nitric acid was selected for further studies.

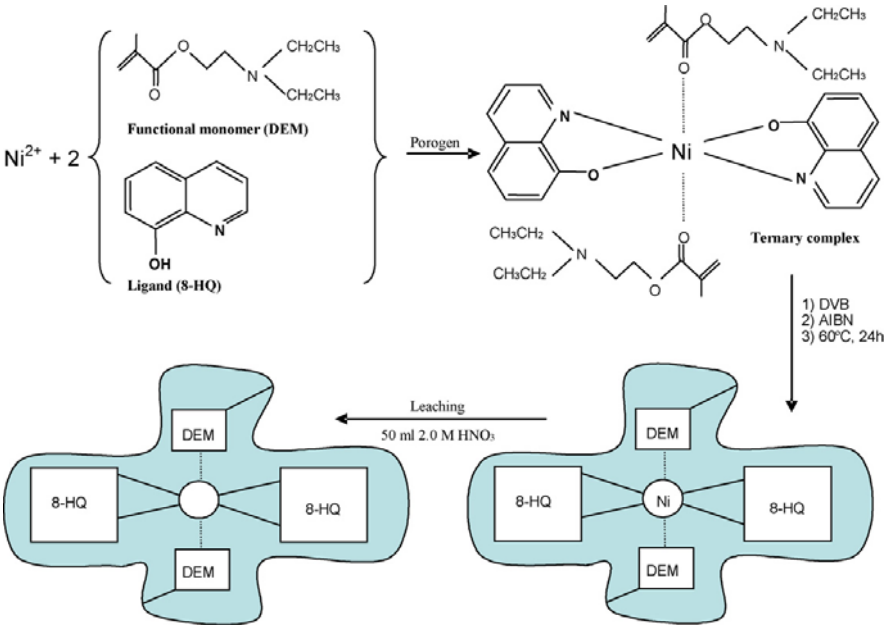

Fig. 2 – Proposed schematic representation of the imprinting process for IIP-4 and IIP-6: dotted lines between Ni(II) ions and DEM show the possibility of a Ni-8-HQ-DEM ternary complex before polymerization.

Similarly and in order to reach the highest pre-concentration factor, a volume of 2.5 mL of the eluting solution (2.0 M nitric acid) was tested. Results after ICP-OES measurement have shown that an efficient nickel elution is reached under both nitric acid volumes (2.5 and 3.0 mL). However, the elution process must be done in two steps. Quantitative nickel analytical recoveries were only obtained if nickel is eluted subsequently with two 1.5 mL aliquots (3.0 mL of the eluting solution) or 1.25 mL aliquots (2.5 mL of eluting solution). This fact could be attributed to the relatively high flow rate used around 1.5 mL min^{-1} which is the lowest flow rate allowed by the vacuum manifold station. A quantitative nickel elution could be reached using 3 mL at once but at a lower *flow rate*. Since both eluting volumes have led to good analytical recoveries, an eluting volume of 2.5 mL was chosen in order to obtain the highest pre-concentration factor.

3.3.2. *Effect of the load flow rate*

Since a vacuum manifold station was used for IIP-SPE pre-concentration, the vacuum was fixed so that the sample solution as well as the rinsing and eluting solutions passed throughout the polymeric material at the lowest flow rate (around 1.5 mL min^{-1}). Other flow rates were tested and quantitative nickel recoveries were found for flow rates up to 10 mL min^{-1}. Therefore, this flow rate was chosen for further experiments.

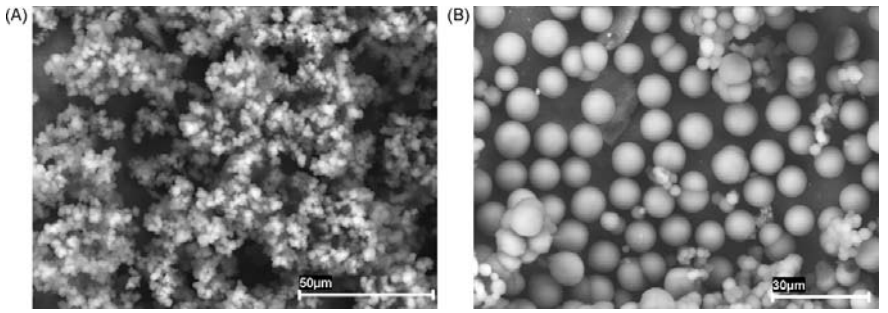

Fig. 3 – Scanning electron microscopy pictures for IIP-6 (A) and NIP-6 (B).

Table 2 – Effect of polymer mass on breakthrough volume.

Sample volume (mL)	Ni analytical recovery (%)[a]	
	100 mg	300 mg
25	91 ± 2	101 ± 3
50	75 ± 5	98 ± 2
75	54 ± 6	97 ± 4
100	43 ± 4	96 ± 4
200	24 ± 6	94 ± 2
250	20 ± 4	92 ± 1

[a] $n = 3$.

3.3.3. Retention capacity

To determine the retention capacity (or sorption capacity) of the polymer (maximum amount of nickel ion retained from 1 g of IIP), 100 mg of polymer were saturated with nickel ion under optimum conditions, by passing subsequently several 3.0 mL aliquots of 200 $\mu g\,mL^{-1}$ Ni(II) solution, and measuring the nickel content in the eluates by ICP-OES. A retention capacity of the polymer was calculated to be 0.023 $mmol\,g^{-1}$. The theoretical retention capacity is 0.28 $mmol\,g^{-1}$; therefore, the calculated retention capacity is around 8% of the theoretical retention capacity. This result agrees with reported retention capacities for MIPs, around a 10% of the theoretical capacity [39].

3.3.4. Breakthrough volume: effect of polymer mass

Two different cartridges were packed with different masses of IIP-6 polymer particles (100 and 300 mg). After adjusting the pH to 8.5 ± 0.5, different volumes (from 25 to 250 mL) of an aqueous solution containing 50 $\mu g\,L^{-1}$ of nickel were passed through the cartridges at a flow rate of 10 $mL\,min^{-1}$. The retained nickel was then eluted with 2.5 mL of 2.0 M nitric acid and determined by ICP-OES. All experiments were performed by triplicate and the found nickel analytical recoveries are listed in Table 2. It can be seen that a polymer mass of 100 mg is not enough to reach quantitative nickel recoveries when using large sample volumes (larger than 25 mL). In this case, the breakthrough volume is 25 mL and when eluting with 2.5 mL of 2.0 M nitric acid, a maximum pre-concentration factor of 10 could only be achieved. However, larger volumes of loading solutions can be used when preparing IIP-SPE cartridges with 300 mg of polymeric material. In such cases, the breakthrough volume is not reached even after loading with 250 mL of sample solutions, and a pre-concentration factor of 100 can be obtained.

As a sensitive detector such as ETAAS is going to be further used to assess nickel in seawater samples, and to achieve a high pre-concentration factor in a reasonable period of time, a sample volume of 100 mL was chosen for a polymer mass of 300 mg, achieving a pre-concentration factor of 40, which is high enough to detect nickel in unpolluted seawater samples by ETAAS or ICP-OES.

3.3.5. Effect of major components from seawater

A set of experiments was carried out in order to observe interactions between the polymeric material and the major metals present in seawater (Na, K, Ca and Mg). A seawater sample was subjected eleven times to the proposed procedure and the concentrations of major ions were determined by ICP-OES. After pre-concentration, values around 30, 2, 10 and 5 $mg\,L^{-1}$ were found for Na, K, Mg and Ca, respectively. These concentrations are very low taking into account the concentration of such elements in seawater (around 11490, 399, 1293 and 413 $mg\,L^{-1}$, for Na, K, Mg and Ca, respectively [40]). Therefore, it can be concluded that salt matrix is efficiently removed by using the IIP-6 and a selective pre-concentration of Ni is achieved.

3.4. Analytical performances for the nickel determination in seawater by IIP-SPE-ETAAS

3.4.1. Calibration: evaluation of matrix effect

Although IIP-SPE implies nickel separation from the seawater matrix, a comparison between calibration in 2.0 M nitric acid and standard addition was established in order to study a possible matrix effect. The standard addition graph was obtained after spiking four aliquots from a mixture of eluates, obtained after pre-concentration of a same seawater sample, with different nickel concentrations (between 0 and 30 $\mu g\,L^{-1}$). The aliquots were measured by ETAAS and 2.0 M nitric acid calibration and standard addition graphs were obtained. The mean and standard deviation for the slopes of three standard addition graphs (0.0046 ± 0.0006 AU L μg^{-1}) and three external 2.0 M nitric acid calibrations (0.0049 ± 0.0004 AU L μg^{-1}) were statistically compared by using the Cochran'C and Bartlett's tests at a 95.0% (comparison of variances), and the ANOVA test (comparison of means). It has been obtained that slopes for external 2.0 M nitric acid calibration and standard addition graphs are statistically comparable so the salt matrix was efficiently removed during the pre-concentration stage. This result agrees with no interaction between Na^+ and K^+ with the polymeric material (Section 3.3.5). Therefore, a simple calibration with nickel standard solution in 2.0 M nitric acid is adequate to perform seawater analysis for nickel. This fact offers a practical advantage so that a tedious and time consuming standard addition technique is not necessary.

Table 3 – Analysis of certified reference materials. Each material was analyzed by triplicate.

SLEW-3		TM-23.3		TM-24	
Certified value ($\mu g\,L^{-1}$)	Found value ($\mu g\,L^{-1}$)	Certified value ($\mu g\,L^{-1}$)	Found value ($\mu g\,L^{-1}$)	Certified value ($\mu g\,L^{-1}$)	Found value ($\mu g\,L^{-1}$)
1.23 ± 0.07	1.30 ± 0.18	5.4 ± 0.6	5.2 ± 0.2	3.5 ± 3.0	3.4 ± 0.2

Most of the reported pre-concentration methods require the establishment of a standard addition graph of the overall SPE and analytical determination procedure [7].

3.4.2. *Sensitivity of the method*

Procedural blanks (i.e. 100 mL of Milli-Q water subjected to the SPE procedure) were performed eleven times and the mean integrated absorbance (0.00906 AU) and standard deviation (0.00894 AU) after ETAAS measurements were obtained. The limit of detection given by LOD = (3 × SD)/*m*, where SD is the standard deviation of eleven procedural blanks, and *m* is the slope of the external 2.0 M nitric acid calibration graph, was calculated to be 137 ng L^{-1} for a pre-concentration factor of 40 and 55 ng L^{-1} for a pre-concentration factor of 100. Similarly, the limit of quantification, given by LOQ = (10 × SD)/*m* (SD and *m* as above), was calculated to be 456 ng L^{-1} for a pre-concentration factor of 40 and 182 ng L^{-1} for a pre-concentration factor of 100. Such LOD and LOQ are low enough to determine trace nickel levels in unpolluted seawater samples, around 0.6 μg L^{-1} [40] and they are similar to those reported by other authors when using ETAAS as a detection technique and Amberlite XAD-4 [41] or Amberlite XAD-2 with Eriochorme blue black R as chelating agent [42], around 100 ng L^{-1}. LODs between 0.06 and 5.0 μg L^{-1} have been summarized by Praveen et al. [27] when using FAAS and different Amberlite sorbents/chelating agents. The use of C18 and ICP-OES detection showed a LOD value of 30 ng L^{-1} [7]. Finally, other sorbent materials based on IIPs have offered LODs of 5.0 μg L^{-1} for FAAS detection [27], 0.3 μg L^{-1} when using ETAAS [29], and 0.16 μg L^{-1} for ICP-OES measurements [30].

3.4.3. *Repeatability and accuracy of the method*

The repeatability of the overall procedure (IIP-SPE and ETAAS determination) was assessed by analyzing the same seawater sample eleven times. A relative standard deviation (RSD) of 6% was achieved for a mean nickel concentration of 1.62 μg L^{-1}, showing good repeatability of the overall procedure.

The accuracy of the method was verified by studying the analytical recovery and by analyzing different certified reference materials offering different salinities (SLEW-3, TM-23.3 and TM-24). Analytical recovery was assessed for three nickel concentration levels, after spiking three different aliquots from the same seawater sample with 1.0, 2.0 and 3.0 μg L^{-1} of Ni. Each nickel concentration was performed by triplicate; thus, analytical recoveries are given as mean ± SD for three independent measurements ($n = 3$). These analytical recoveries are 104 ± 3%, 98 ± 2% and 99 ± 5% for 1.0, 2.0 and 3.0 μg L^{-1} of Ni, respectively. It can be concluded that complete analytical recovery (within the 95–105% range) was reached for all the nickel concentration levels.

SLEW-3 (estuarine water), TM-23.3 (lake water) and TM-24 (lake water) certified reference materials were analyzed in triplicate using a sample volume of 25 mL (pre-concentration factor of 10). Results, listed in Table 3, reveal good agreement between found concentrations and certified values for the three certified reference materials. This fact has been verified after applying the *t*-test for means comparison.

3.5. *Application to real seawater samples*

The optimized IIP-SPE-ETAAS method has been applied to five surface estuarine water samples from the Ría de Muros estuary (north-western Spain). All samples were sampled at the same sampling point in five different days. In addition, the method was applied to forty-five surface seawater samples from the A Coruña harbour (north-western Spain). In this case, samples were sampled at three different sampling points in fifteen different days. Each sample was subjected three times to the optimized procedure (pre-concentration factor of 40). Nickel concentrations in estuarine waters were between 1.21 ± 0.0442 and 1.70 ± 0.0350 μg L^{-1}, while nickel concentration between 0.480 ± 0.0103 and 6.52 ± 0.0305 μg L^{-1} were measured in seawaters from A Coruña harbour.

4. Conclusions

Nickel IIPs synthesized in the presence of DEM and 8-HQ have shown recognition capacities for nickel as well as quantitative pre-concentration of nickel(II) from seawater samples. Pre-concentration by solid-phase extraction with IIP-6 particles results in a pre-concentration factor of 100 (250 mL of seawater sample and 2.5 mL of eluate), which offers a limit of detection of 50 ng L^{-1} by using ETAAS as a selective detector. The synthesized polymeric material has not offered affinity for major elements in seawater samples such as sodium and potassium, removing efficiently the salt matrix of seawater. This fact has been verified after statistical comparison of nickel aqueous standard calibrations in 2.0 M nitric acid and standard addition calibrations. Therefore, nickel measurements can be directly carried out using an aqueous calibration in 2.0 M nitric acid, being a fast method when coping with large number of samples. Finally, the IIP-SPE combined with ETAAS determination has offered accurate results for the analysis of nickel in lake water (low salinity) and estuarine water (high salinity).

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Inductively coupled plasma–optical emission spectrometry/mass spectrometry for the determination of Cu, Ni, Pb and Zn in seawater after ionic imprinted polymer based solid phase extraction

Jacobo Otero-Romaní[a], Antonio Moreda-Piñeiro[a], Pilar Bermejo-Barrera[a,*], Antonio Martin-Esteban[b]

[a] Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n, 15782 Santiago de Compostela, Spain
[b] Department of Environment, National Institute for Agriculture and Food Research (INIA), Carretera de A Coruña, Km 7,5, 28040 Madrid, Spain

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ABSTRACT

The capabilities of a synthesized ionic imprinted polymer (IIP), originally prepared for Ni recognition/pre-concentration from seawater, have been evaluated for other trace elements pre-concentration. The polymer has been synthesized by the precipitation polymerization technique using a ternary pre-polymerization complex formed by the template (Ni), the monomer (2-(diethylamino) ethyl methacrylate, DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ). Since the complexing agent (8-HQ) is trapped into the polymeric matrix, but is not linked to the polymer chains, specific interactions between the functional groups (present in the monomer and the complexing agent) and other trace elements rather than Ni may occur.

Results have shown that the IIP offers imprinting properties for the template (Ni(II)) and also for Cu(II), Pb(II), Zn(II), As(V) and Cd(II), with analytical recoveries close to 100% for all elements except for As(V) and Cd(II) (around 70%), whereas the non-imprinted polymer (NIP) did not show affinity for any trace element. In addition, the polymer does not interact with alkaline or alkaline-earth metals, so Na, K, Mg and Ca from the seawater salt matrix could be effectively removed. Variables affecting the IIP-solid phase extraction (SPE) process (pH, load flow rate and concentration and volume of the eluting solution) were completely studied. Inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS) have been used as multi-element detectors.

Acidified seawater samples must only be treated to fix an alkaline pH (8.5 ± 0.5) and passed through IIP-SPE cartridges. After seawater sample loading (250 mL), analytes were eluted with 2.5 mL of 2.0 M nitric acid, offering a pre-concentration factor of 100. Therefore, the limits of detection (LODs) of the method were 0.14, 0.15, 0.18 and 0.03 $\mu g\,L^{-1}$, for Ni, Cu, Pb and Zn, respectively, when using ICP-OES detection and 0.0022, 0.0065, 0.0040 and 0.009 $\mu g\,L^{-1}$, for Ni, Cu, Pb and Zn, respectively, for ICP-MS detection. Accuracy of the method was assessed by analyzing SLEW-3 (estuarine water), and TM-23.3 and TM-24 (lake water) certified reference materials.

1. Introduction

The protection of marine and continental waters against contamination has increased in recent years to allow adequate conditions for aquatic life and a responsible use of such natural resources. The European Union has therefore established several Directives on the quality of continental and marine waters and for marine ecosystems. These European Directives limit the levels of some trace elements such as Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in seawater used for mollusk production [1]. Therefore, the development of fast and reliable analytical methods to assess trace elements in water is a current topic. This fact is especially important for seawater analysis because of the high salt content which is a source of spectral and matrix interferences [2,3] and because most of the target metals are present at very low levels [4].

Solid phase extraction (SPE) is, by far, one of the most used sample pre-treatments for complex matrices such as seawater, mainly because trace analyte pre-concentration and sample matrix (salts) removal are achieved at time [5]. Bonded silica sorbents are used in typical SPE procedures for organic compound pre-concentration and also for trace elements [6–8]. However, it has been reported that interfering compounds are often co-extracted with the target

* Corresponding author.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).

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analytes and a matrix effect can be observed using these conventional sorbents [6]. Therefore, more selective systems for separation of trace elements are required and the development or synthesis of new sorbents has increased in recent years [9]. High selectivity can be expected from sorbents such as inmunosorbents (ISs) and molecularly imprinted polymers (MIPs) [10]. However, the main advantage of MIPs over ISs is the fast and less expensive synthesis and the high degree of molecular recognition. Synthesized sorbents in the presence of ions, instead of molecules, as templates generate ionic imprinted polymers (IIPs), which offer all the benefits derived from MIPs and a high capacity for recognizing ions.

Ionic imprinting polymerization is based on the preparation of a highly cross-linked polymer around an ion (the template) in the presence of a suitable monomer (vinylated reagent) or in the presence of a monomer and an auxiliary/complexing non-vinylated reagent [11,12]. As recently reviewed by Rao et al. [12], there are four different approaches for the synthesis of metal ion imprinted polymers, viz. (i) linear chain polymers carrying metal-binding groups being cross-linked with a bifunctional reagent; (ii) chemical immobilization by preparation of binary complexes of metal ions with ligands having vinyl groups, isolation and then polymerization with matrix-forming monomers; (iii) surface imprinting conducted on an aqueous–organic interface; (iv) trapping of non-vinylated chelating ligand via imprinting of binary/ternary mixed ligand complexes of metal ions with non-vinylated chelating agent and vinyl ligand. The first three approaches use vinylated reagents, which form complexes with the template (metal ion) and can polymerize through the vinyl groups. Therefore, the complexing ligand is chemically immobilized in the polymeric matrix. The main drawback of these approaches is the fact that complexing ligands showing vinyl groups are scarce and are not available, so the synthesis of these complexing monomers must be performed in the laboratory. Some examples of these complexing monomers are those based on amino acids such as methacryloylhistidine (MAH) [9,13], and N-methacryloly-(l)-cysteinemethylester (MAC) [14–16], or N-methacryloyl-L-glutamic acid (MAGA) [17], which have been widely used for recognizing Al [17], Cd [14,15], Cu [13], Fe [16] and Ni [9]. Other ligands such as (2Z)-N,N′-bis(2-aminoethylic)but-2-enediamide for Cd [18] and o-phenylenediamine for Se [19] have also been reported as bifunctional monomers.

The use of specially synthesized vinylated ligands can be avoided when using the trapping technique. In this case, a ternary monomer non-vinylated ligand–metal complex is formed; therefore, the number of useful ligands is large because most of the metal complexing agents are not vinylated reagents. After polymerization, the non-vinylated ligand is not chemically bonded to the polymer chains, but instead is trapped inside the polymeric matrix. By using the trapping approach, different non-vinylated reagents such dimethylglyoxime for recognizing palladium [20], diazoaminobenzene [21] for recognizing mercury, cetyltrimethyl ammonium bromide [22] for recognizing Cd and Zn, and derivatives of quinoline, mainly 5,7-dichloroquinoline-8-ol for lanthanides [23–26], Pd [27], U [28–30] and Co and Ni [31] have been successfully applied.

The aim of the current work has been the study of the capabilities of a synthesized ionic imprinted polymer, originally prepared for Ni recognition/pre-concentration from seawater, for other trace elements pre-concentration. The polymer has been synthesized by the precipitation polymerization technique using a ternary pre-polymerization complex formed by the template (Ni), the monomer (2-(diethylamino) ethyl methacrylate, DEM) and a non-vinylated chelating agent (8-hydroxyquinoline, 8-HQ). Inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS) have been used as multi-element detectors.

2. Experimental

2.1. Instrumentation

Optima 3300 DV inductively coupled plasma atomic emission spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with an autosampler AS 91 (PerkinElmer) and a Gem-Cone cross-flow nebulizer type (PerkinElmer) was used for multi-elemental determinations. 820-MS inductively coupled plasma quadrupole mass spectrometer (Varian, Mulgrave, Australia) equipped with an SPS3 autosampler (Varian) and a MicroMist nebulizer type (Varian). Temperature-controllable incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. Vacuum manifold station (Waters, Milford, MA, USA) connected to a vacuum pump (Millipore Co., Bedford, MA, USA) was used for SPE. IIPs were packed into 5 mL syringes (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA). ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Ultra-pure water of resistivity 18 MΩ cm obtained from a Milli-Q purification device (Millipore Co.) was used to prepare all the solutions. 70% nitric acid and analytical grade $NiCl_2 \cdot 6H_2O$ were purchased from Panreac (Barcelona, Spain). Single standard solutions ($1000\,mg\,L^{-1}$) of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn were from Merck (Darmstadt, Germany). Ammonia 25% (v/v), ammonium chloride, and 8-hydroxyquinoline were purchased from Merck. HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). 2-(diethylamino) ethyl methacrylate (DEM) used as monomer was from Sigma–Aldrich (Steinheim, Switzerland). Divinylbenzene-80 (DVB) from Sigma–Aldrich was treated in order to remove the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing around 0.5 g of neutral alumina (Sigma–Aldrich). 2,2′-azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at −20 °C after dissolving the reagent in methanol (Merck) at 50–60 °C. After purification, this reagent was stored at 4 °C. Estuarine seawater (SLEW-3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM-24 and TM-23.3) certified reference materials were purchased from the National Water Research Institute of Canada. All glass and plastic material was rigorously cleaned and kept into 10% (v/v) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before being used.

2.3. Seawater collection

Seawater samples (1 L) were collected from the Ría de Muros-Noia estuary (north-western Spain) in pre-cleaned high density polyethylene bottles. After collection, seawater samples were filtered through 0.45 μm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then stored at low temperature.

2.4. ICP-OES/MS measurements

Multi-element determinations (As, Cd, Cu, Ni, Pb and Zn) were performed by ICP-OES (axial configuration) using the operating conditions and emission wavelength lines given in Table 1. Determinations were performed by using aqueous standards in 2.0 M nitric

Table 1
Operating ICP-OES conditions.

General		
	Radiofrequency power/W	1300
	Sample uptake rate/mL min^{-1}	1.5
	Stabilization delay/s	45
	Number of replicates	4
	Integration time/s	5
	Nebulizer type	Cross-flow
	Axial view	
Gas flows/L min^{-1}		
	Plasma	15.0
	Auxiliary	0.5
	Nebulizer	0.8
Detection wavelengths/nm		
	Cu	327.393
	Ni	231.604
	Pb	224.688
	Zn	213.857

acid covering metal concentrations within the 0–0.25 mg L^{-1} range for As, Cd, Cu, Ni and Pb, and between 0 and 1.0 mg L^{-1} for Zn.

Similarly, As, Cd, Cu, Ni, Pb and Zn were measured by ICP-MS under operating conditions listed in Table 2. The use of H_2 in the collision cell at a flow rate of 80 mL min^{-1} gave the best sensitivity as well as minimised possible polyatomic interferences for the target elements [32]. This flow was optimized by monitoring the counts for a 2.0 M nitric acid eluate containing the target elements. Finally, determinations were performed by using aqueous standards in 2.0 M nitric acid with metal concentrations from 0 to 0.25 mg L^{-1} (As, Cd, Cu, Ni and Pb) and Zn concentrations from 0 to 1.0 mg L^{-1}.

Table 2
Operating ICP-MS conditions.

General		
	Radiofrequency power/W	1400
	Sample uptake rate/r.p.m.	3.0
	Stabilization delay/s	35
	Number of replicates	3
	Integration time/s	5
	Nebulizer type	MicroMist
Gas flows/L min^{-1}		
	Plasma	17.0
	Auxiliary	1.65
	Sheath	0.27
	Nebulizer	0.99
Torch alignment/mm		
	Sampling death	7.0
Ion optics/V		
	First extraction lens	−32
	Second extraction lens	−164
	Third extraction lens	−231
	Corner lens	−206
	Mirror lens right	25
	Mirror lens left	24
	Mirror lens bottom	27
	Entrance lens	3
	Fringe Bias	−4.9
	Entrance plate	−3.4
	Pole bias	0
CRI/mL min^{-1}		
	Skimmer gas source	H_2
	Sampler gas source	OFF
	Skimmer flow	80
	Sampler flow	0
Mass-to-ratio		
	Cu	63
	Ni	60
	Pb	208
	Zn	66

2.5. Synthesis of nickel ionic imprinted polymer particles

0.0334 g of $NiCl_2{\cdot}6H_2O$, 56.5 μL of DEM and 0.0817 g of 8-HQ were mixed in 12.5 mL of porogen (3:1 acetonitrile:toluene). This solution was stirred for 5 min and then filtrated. Finally, 500 μL of DVB (cross-linker) and 41.3 mg of AIBN (initiator) were added, the glass tubes purged with N_2 and immediately sealed just before thermal induction of the precipitation polymerization. The temperature was ramped from room temperature to 60 °C over 2 h, and then maintained at 60 °C for further 24 h. Always, a low stirring rate of 33 r.p.m. was used. The polymer was vacuum filtered, washed with acetonitrile and then oven-dried overnight at 40 °C. Finally, the polymer (around 300 mg) was packed into 5 mL cartridges between Teflon frits. It should be mentioned that the polymer was synthesized in the presence of a double amount of 8-HQ with respect to DEM. This gives a Ni/DEM/8-HQ molar ratio of 1/2/4. The mass of polymer obtained after polymerization was 0.300 g and the efficiency of the polymerization process, taking into account a theoretical amount of synthesized polymer of 0.5 g, was 60%. The template (Ni(II) ions) was removed from the polymer particles by extensive washing with 2.0 M nitric acid (5.0 mL aliquots). Negligible nickel (template) concentrations by ICP-MS in the washing/filtrate solutions were found after passing 50 mL of the washing solution. Blank polymer particles (non-imprinted polymer, NIP) were also prepared in the same way than IIP, but without the template. The NIP was then subjected to the same washing pretreatment as described above.

The synthesized materials (IIP and NIP) were characterized by scanning electron microscopy (SEM), energy dispersive X-ray fluorescence (EDXRF) and microanalysis (elemental H, C, N and O composition). This information is given elsewhere [35].

2.6. IIP-solid phase extraction

Multi-element aqueous standard solutions were prepared in 100 or 250 mL of 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at pH 8.5 ± 0.5. Similarly, 100 or 250 mL of acidified seawater samples were treated with volumes of a 5.0 M ammonia solution (with the 100–500 μL range), to readjust the pH to 8.5 ± 0.5. Then, the solutions were passed through cleaned and conditioned IIP cartridges at a flow rate of 3.0 mL min^{-1} by using a vacuum manifold station. The cartridges were then rinsed with 2.5 mL of the 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at pH 8.5 ± 0.5, and then, the retained ions were subsequently eluted with two 1.25 mL aliquots of 2.0 M nitric acid solution at a flow rate of 1.5 mL min^{-1}. A pre-concentration factor of 40 or 100 was achieved under these operating conditions. After elution, the IIPs were treated with 10 mL of Milli-Q water and then conditioned by passing 10 mL of the 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at the working pH (8.5).

Blanks of the procedure were also obtained by applying the SPE procedure above described. In this case, around 1 L of Milli-Q water was acidified by adding 1.0 mL of concentrated nitric acid (pH lower than 2), and then 100 mL aliquot samples were treated with the buffer solution to fix the pH 8.5 ± 0.5, and were subjected to the SPE procedure.

3. Results and discussion

3.1. Optimization of trace elements IIP-SPE from seawater

3.1.1. Effect of pH

A set of experiments was carried out by applying the preconcentration procedure (Section 2.6) to 100 mL aliquots of an aqueous solution containing the template, Ni(II), and other metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Pb, Sn, Ti, V and Zn) at 50 μg L^{-1} each

one. The same experiments were carried out by using the NIP. The different pHs tested (from 4.0 to 9.0) were fixed by using 0.1 M/0.1 M NH_4Cl/NH_3 buffer solutions.

Three replicate SPE were performed at each pH and after ICP-OES detection, the polymeric material offered specific interactions for Ni and also for Cu(II), Pb(II), Zn(II), As(III) and Cd(II) since the analytical recoveries for the non-imprinted polymer are quite lower than when using the IIP for most of the tested pHs. Other trace elements were not specifically retained at any pH.

Fig. 1 shows the effect of the pH on the analytical recovery of Ni, Cu, Pb, Zn, As and Cd after IIP/NIP-SPE. It can be observed that Ni and Zn are only quantitatively retained in the IIP at pHs in-between 8.0 and 9.0, whereas Cu and Pb are quantitatively retained at all tested pHs. As and Cd show analytical recoveries near 70% at all pHs tested.

The fact that other ions in addition to the template (Ni(II)) interact with the specific cavities formed for nickel must be explained keeping in mind the presence of the non-vinylated agent (8-HQ). After polymerization, this agent is trapped into the polymeric matrix and the specific cavities formed for nickel during the polymerization are influenced by the presence of 8-HQ which can interact with other ions, although the shape and/or size of such ions was different to the template. The pre-concentrating capacities of the synthesized polymer for Ni(II), Cu(II), Pb(II) and Zn(II) are in agreement with other pre-concentration studies based on resins-immobilized 8-HQ, such as those reported by Howard et al. for silica-immobilized 8-HQ [33], and by Askun et al. for 8-HQ anchored poly(styrene-divinylbenzene) microbeads [34]. For both supports, the same divalent trace elements (Cu, Ni and Zn [33], and Pb, Cd, Ni and Co [34]) were selectively pre-concentrated. This selectivity must be related to a metal complexation with the immobilized 8-HQ because many elements are complexed with 8-HQ in solution [6]. Therefore, the "flexibility" of the imprinting cavities, attributed to 8-HQ, gives the possibility of using this IIP for the selective pre-concentration of a certain number of elements: Cu, Ni, Pb and Zn. From the previous studies (Fig. 1), a compromise pH of 8.5 ± 0.5 was chosen to achieve the highest analytical recovery for this group of elements.

3.1.2. Effect of the load flow rate

Different experiments were performed in order to find the optimum load flow rate for the sample solutions. Different aliquots from the same seawater sample were passed subsequently at 1.5, 3.0, 6.0 and 12 mL min^{-1}. The flow rates were fixed by using a vacuum manifold station. Results have shown the same As, Cd, Ni and Zn concentrations for all flow rates tested; however, Cu and Pb concentrations decrease for flow rates larger than 3 mL min^{-1}. In order to achieve the highest analytical recovery in the lowest period of time, a 3 mL min^{-1} load flow rate was chosen, and used for further experiments.

3.1.3. Effect of nitric acid volume/concentration for elution

Different experiments were performed in order to find the optimum nitric acid concentration/volume for the eluting solution. After sample loading and rinsing (Section 2.6), the retained analytes were eluted by passing volumes of 2.5 mL of nitric acid at concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 M at a flow rate of 1.5 mL min^{-1}. After measuring by ICP-OES against an aqueous calibration in 1.0, 2.0, 3.0, 4.0 or 5.0 M nitric acid, quantitative analytical recoveries were obtained for all cases; thus, an eluting solution of 2.0 M nitric acid was selected for further studies.

Regarding the volume of the eluting solution, volumes of 2.5 and 3.0 mL of a 2.0 M nitric acid solution were tested. Experiments in triplicate at a fixed flow rate of 1.5 mL min^{-1} have shown that an efficient metal elution is reached under both nitric acid volumes, although the elution process must be done in two steps. Therefore, analytes were eluted subsequently with two 1.5 mL aliquots (3.0 mL of the eluting solution) or 1.25 mL aliquots (2.5 mL of the eluting solution). In order to achieve the highest pre-concentration factor, an eluting volume of 2.5 mL was chosen (pre-concentration factor of 100 for a seawater sample volume of 250 mL).

3.2. Effect of major components from seawater

Different experiments were carried out to observe interactions between the polymeric material and the major metals present in seawater (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}). To check possible interaction of the major cations, a seawater sample was subjected eleven times to the proposed procedure and the concentrations of major ions were determined by ICP-OES. After pre-concentration (factor pre-concentration of 100), values around 30, 2, 10 and 5 mg L^{-1} were found for Na, K, Mg and Ca, respectively. These concentrations are very low taking into account the concentration of such elements in seawater (around 11490, 399, 1293 and 413 mg L^{-1}, for Na, K, Mg and Ca, respectively [4]).

To check possible retention of chloride and sulphate, a seawater sample was subjected eleven times to the proposed procedure (pre-concentration factor of 100) and the nitric acid eluates were measured by ICP-MS (m/z ratios of 32 and 35 for sulphur and chlorine, respectively). Negligible signals for both analytes were obtained, which means they are not retained in the polymeric matrices or that potential polyatomic interferences are avoided by using collision cell. Therefore, it can be concluded that salt matrix is efficiently removed while a selective pre-concentration of Ni, Cu, Pb and Zn is achieved.

3.3. Cross-reactivity

Different experiments were conducted in order to evaluate the cross-reactivity among nickel and other selected trace elements (Al, As, Cd, Cr, Cu, Mn, Pb, Sn, Ti, V, and Zn). 100 mL aliquots of an aqueous standard solution at pH of 8.5 ± 0.5 containing 50 μg L^{-1} of nickel and other trace metals were subjected three times to the optimized SPE procedures by using cartridges prepared with IIP and NIP materials. After elution and ICP-OES measurements, parameters such as extraction efficiency, distribution ratio (D) and selectivity coefficient ($S_{Ni/M}$), defined as shown in Table 3, were calculated. As it was expected, results (Table 3) show that the polymeric material (IIP) does not discriminate between Ni, Cu, Pb and Zn, as they remain linked to the polymer (percent extraction higher than 99%) and only detach after acidic elution. It can be concluded that the polymer offers imprinting properties for Ni (the template) and also for Cu, Pb and Zn whereas the NIP material did not show affinity for Ni, Cu, Pb or Zn (percent extraction within 10–27%), indicating that those specific cavities formed for nickel during the IIP polymerization are responsible of Ni, Cu, Pb and Zn retention. The IIP material is specific for Ni, Cu, Pb and Zn, and other transition metals (Cr, Sn, Ti, V, As, Cd, Mn and Al) are not retained.

3.4. Analytical performances for the IIP-SPE–ICP-OES/MS procedures

3.4.1. Calibration. Evaluation of matrix effect

Three different calibration graphs were performed: (1) external aqueous calibrations in 2.0 M nitric acid; (2) standard addition calibrations, obtained after spiking a pool of nitric acid eluates from different 100 mL aliquots from the same seawater sample with the target elements; and (3) standard addition calibrations prepared following the IIP-SPE pre-concentration procedure (100 mL seawater sample spiked with 1.0, 2.0 and 4.0 μg L^{-1} for As, Cd, Cu, Ni and Pb and 5, 10 and 20 μg L^{-1} for Zn, and subjected to the IIP-SPE procedure (Section 2.6)). External aqueous calibrations and standard

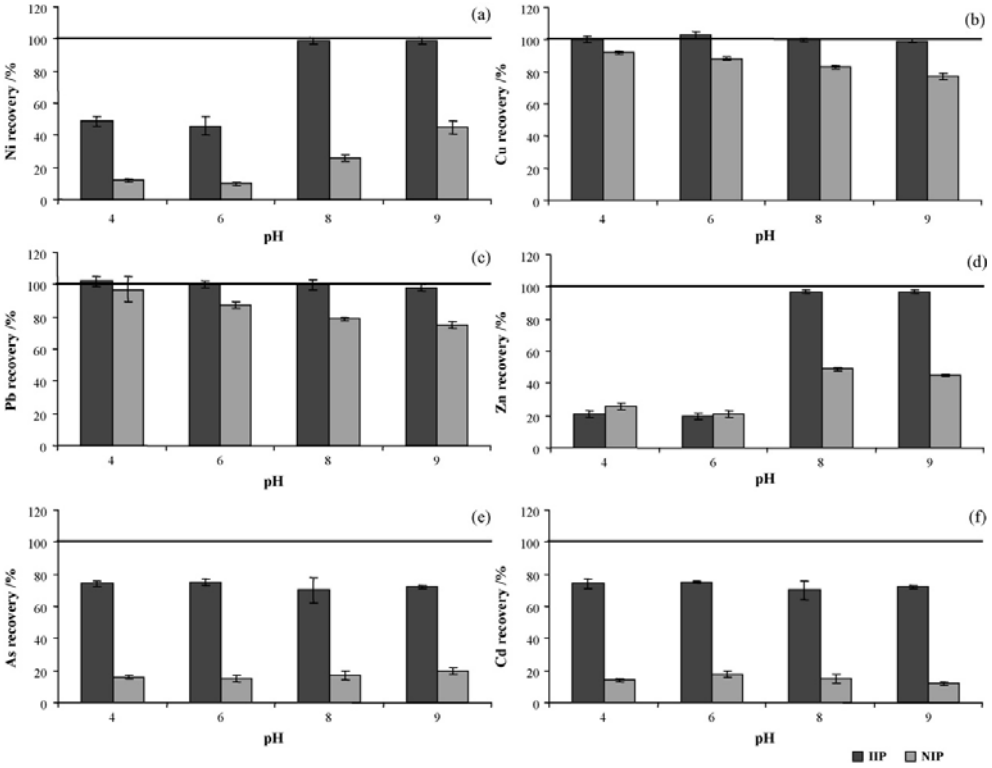

Fig. 1. Effect of pH on nickel (a), copper (b), lead (c), zinc (d), arsenic (e) and cadmium (f) analytical recovery after solid phase extraction with an ionic imprinted polymer (IIP) and a blank or non-imprinted polymer (NIP).

addition calibrations were tested covering metal concentrations between 0 and 0.25 mg L^{-1} (As, Cd, Cu, Ni and Pb) and between 0 and 1.0 mg L^{-1} (Zn).

For all cases, three different external aqueous calibrations, standard addition calibrations and "procedure" addition calibrations were performed on different days. The mean and the standard deviation of the slopes (ICP-OES and ICP-MS) are given in Table 4. After statistical comparison of the means by the multiple range test (95% confidence range), it has been obtained that slopes for external aqueous calibration in 2.0 M nitric acid and standard addition calibrations are statistically comparable for all elements when using either ICP-OES and ICP-MS. Therefore, it can be concluded that salt matrix is efficiently removed during the pre-concentration stage. This result agrees with the fact that there is no interaction between Na and K with the polymeric material [35]. In addition, slopes of the external aqueous calibration in 2.0 M nitric acid and the "procedure" addition calibrations are also statistically similar (95% confidence range), for those elements which were quantitatively retained by the polymer (Ni, Cu, Pb and Zn). The slopes for other elements (As and Cd), which show specific interactions but non-quantitative retention, were not statistically comparable. The same results have been obtained when comparing the standard addition calibrations and the standard addition calibration prepared following the IIP-SPE pre-concentration procedure. Therefore, an external aqueous calibration in 2.0 M nitric acid can be used to determine Ni, Cu, Pb and Zn in seawater after IIP-SPE, but a standard addition calibration prepared following the IIP-SPE pre-concentration procedure is needed for the quantitative determination of As and Cd in the nitric acid eluates after IIP-SPE.

The feasibility of external aqueous calibration offers a practical advantage so that tedious and time consuming standard addition techniques are not necessary. It should be mentioned that most of the reported pre-concentration methods require the establishment of a standard addition graph of the overall SPE and analytical determination procedure [8], so the synthesized polymer is advantageous for Ni, Cu, Pb and Zn pre-concentration/determination in seawater.

3.4.2. Sensitivity of the method

The limits of detection and quantification were calculated according to LOD = (3S.D.)/m and LOD = (10S.D.)/m, where S.D. is the standard deviation of eleven measurements of a procedural blank (acidified Milli-Q water treated as a sample) and m is the slope of the external 2.0 M nitric acid calibration graph. Values after a pre-concentration factor of 100 are listed in Table 5. Such LODs and LOQs are low enough to determine Ni, Cu, Pb and Zn levels in unpolluted seawater samples. These values are similar to those reported by other authors when using ICP-MS (0.01 μg L^{-1} for Ni using Amberlite XAD-4 immobilized butane-2,3-dione bis(N-pyridimoacetylhydrazone) [36], and from 0.0001 μg L^{-1} (Pb) to 0.001 μg L^{-1} (Ni) for Chelex 100 down-sized packed mini-columns [37]), ICP-OES (values from 0.02 to 1.13 μg L^{-1} for the use of C18 [6]) and ETAAS (0.10 μg L^{-1} for Ni using Amberlite XAD-4 [38] or Amberlite XAD-2 with Eriochorme blue black R as chelating agent [39]). Finally, other sorbent materials based on IIPs have offered LODs for

Table 3
Extraction (%), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$) for IIP and NIP.

Element	Extraction (%)[a]	Distribution ratio (D)[b]	Selectivity coefficient ($S_{Ni/M}$)[c]
Ionic imprinted polymer (IIP)			
Ni	99	99	–
Cu	99	99	1.0
Pb	99	99	1.0
Zn	99	99	1.0
Cr	80	4.0	25
Sn	78	3.5	28
Ti	80	4.0	25
V	21	0.3	372
As	72	2.6	38
Cd	71	2.4	40
Mn	16	0.2	520
Al	24	0.3	314
Non-ionic imprinted polymer (NIP)			
Ni	10	0.1	–
Cu	23	0.3	0.4
Pb	27	0.4	0.3
Zn	19	0.2	05
Cr	75	3.0	0.04
Sn	73	3.0	0.04
Ti	79	3.8	0.03
V	21	0.3	0.42
As	14	0.2	0.68
Cd	14	0.2	0.68
Mn	12	0.1	0.81
Al	21	0.3	0.42

A_1 = Amount of metal ion in aqueous solution at equilibrium; A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium. A_T = Total amount of metal ion used in extraction. D_{Ni} = Distribution ratio for Ni; D_M = Distribution ratio for M (M = Cu, Pb, Zn, Cr, Sn, Ti, V, As, Cd, Mn and Al).

[a] $\% = (A_2/A_T) \times 100$.

[b] $D = (A_2/A_1)$.

[c] $S_{Ni/M} = D_{Ni}/D_M$.

Ni of 5.0 μg L^{-1} (FAAS detection) [31], 0.3 μg L^{-1} [9] or 0.18 μg L^{-1} [35] when using ETAAS, and 0.16 μg L^{-1} for ICP-OES measurements [40].

3.4.3. Repeatability and accuracy of the method

The repeatability of the overall procedure was assessed by analyzing a seawater sample eleven times. The IIP-SPE pre-concentration procedure implies a pre-concentration factor of 40 (100 mL of seawater sample). The percent relative standard deviation (RSD) values were 4, 7, 8 and 7% for Ni, Cu, Pb and Zn determination by ICP-OES, while RSDs of 6% for Ni and 5% for Cu, Pb and Zn were achieved by using ICP-MS.

Table 4
Means and standard deviations (mean ± S.D.) for aqueous calibration in 2.0 M nitric acid, standard addition and standard addition calibrations prepared following the IIP-SPE pre-concentration procedure (N = 3).

	Aqueous calibration	Standard addition	Standard addition calibration following the IIP-SPE
ICP-OES[a]			
Ni	6.8 ± 0.1	6.6 ± 0.1	6.6 ± 0.2
Cu	63 ± 2	62 ± 2	61 ± 1
Pb	3.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.2
Zn	10 ± 0.2	10 ± 0.1	9.9 ± 0.1
As	6.5 ± 0.3	6.1 ± 0.2	3.7 ± 0.2
Cd	6.1 ± 0.1	6.0 ± 0.1	3.7 ± 0.2
ICP-MS[b]			
Ni	545 ± 35	530 ± 27	496 ± 27
Cu	1394 ± 85	1385 ± 71	1291 ± 111
Pb	74754 ± 587	71825 ± 1115	70078 ± 4192
Zn	9137 ± 255	9195 ± 366	9049 ± 709
As	364 ± 4	362 ± 14	63 ± 1
Cd	3544 ± 287	3467 ± 167	2225 ± 192

[a] L mg^{-1}.

[b] L μg^{-1}.

Table 5
LOD and LOQ of the methods.

	LOD/μg L^{-1}	LOQ/μg L^{-1}
ICP-OES		
Ni	0.14	0.47
Cu	0.15	0.50
Pb	0.18	0.60
Zn	0.03	0.11
ICP-MS		
Ni	0.0022	0.0073
Cu	0.0065	0.0217
Pb	0.0040	0.0133
Zn	0.009	0.031

Analytical recovery was assessed for three concentration levels, after spiking different aliquots from the same seawater sample with 1.0, 2.0 and 4.0 μg L^{-1} of Ni, Cu, Pb, and with 5, 10 and 20 μg L^{-1} of Zn. Each concentration level (low, medium and high) was tested by triplicate; thus, analytical recoveries (Table 6) are given as mean ± S.D. for three independent measurements. It can be concluded that good analytical recovery (within the 95–105% range) are reached for all the target elements and concentration levels.

The analysis of estuarine water (SLEW-3) and lake water (TM-23.3 and TM-24) was performed to assess accuracy. Each CRM was subjected to the IIP-SPE procedure three times using a sample volume of 100 mL (pre-concentration factor of 40). Then, determinations were carried out by ICP-OES and ICP-MS. Results, listed in Table 7, reveal good agreement between concentrations found and the certified values for the three certified reference materials. This fact has been proved after applying a t-test (95% confidence range) for comparing the mean values.

3.4.4. Study of the lifetime of the Ni-IIP support

Three different Ni-IIP cartridges were tested in order to know the number of sequential solid phase extraction (loading/elution cycles) that can be performed without losses on pre-concentration efficiency. Experiments were carried out by treating estuarine seawater samples and aqueous standard solutions containing 1.0 μg L^{-1} of Ni, Cu and Pb, and 5.0 μg L^{-1} of Zn. After each five loading/elution cycles with estuarine seawater samples an aqueous standard solution was pre-concentrated and the analytical recovery calculated. Results have shown that Cu, Pb and Zn are not efficiently retained/elute after around twenty uses (analytical recoveries lower than 95%). However, quantitative analytical recoveries were obtained for Ni up to 40 SPE cycles. It must be said that the lifetime of the polymer is larger for the analyte template (Ni) than for other target elements (Cu, Pb and Zn). Therefore, the

Table 6
Analytical recovery of the methods (N = 3).

	Low[a]	Medium[b]	High[c]
ICP-OES			
Ni	105 ± 7	103 ± 3	102 ± 2
Cu	95 ± 3	98 ± 3	100 ± 2
Pb	93 ± 2	98 ± 1	104 ± 4
Zn	99 ± 3	99 ± 6	104 ± 5
ICP-MS			
Ni	103 ± 2	104 ± 1	100 ± 1
Cu	95 ± 5	103 ± 2	99 ± 1
Pb	95 ± 2	105 ± 1	101 ± 3
Zn	108 ± 2	109 ± 1	101 ± 2

[a] 1.0 μg L^{-1} for Ni, Cu and Pb; 5 μg L^{-1} for Zn.

[b] 2.0 μg L^{-1} for Ni, Cu and Pb; 10 μg L^{-1} for Zn.

[c] 4.0 μg L^{-1} for Ni, Cu and Pb; 20 μg L^{-1} for Zn.

Table 7
Analysis of certified reference materials. Each material was analyzed by triplicate.

	ICP-OES					
	SLEW-3		TM-23.3		TM-24	
	Certified/μg L^{-1}	Found/μg L^{-1}	Certified/μg L^{-1}	Found/μg L^{-1}	Certified/μg L^{-1}	Found/μg L^{-1}
Ni	1.2 ± 0.1	1.3 ± 0.1	5.4 ± 0.6	5.7 ± 0.3	3.5 ± 3.0	3.3 ± 0.3
Cu	1.6 ± 0.1	1.4 ± 0.1	9.1 ± 0.6	9.2 ± 1.0	8.0 ± 4.1	7.2 ± 0.5
Pb	0.009 ± 0.001	< LOD	3.2 ± 0.3	3.3 ± 0.6	7.3 ± 2.6	6.9 ± 1.8
Zn	0.20 ± 0.04	0.20 ± 0.02	–[a]		5.4 ± 2.3	5.1 ± 3.0

	ICP-MS			
	SLEW-3		TM-23.3	
	Certified/μg L^{-1}	Found/μg L^{-1}	Certified/μg L^{-1}	Found/μg L^{-1}
Ni	1.2 ± 0.1	1.3 ± 0.04	5.4 ± 0.6	5.8 ± 0.1
Cu	1.6 ± 0.1	1.6 ± 0.02	9.1 ± 0.6	9.6 ± 0.7
Pb	0.009 ± 0.001	<LOD	3.2 ± 0.3	3.5 ± 0.3
Zn	0.20 ± 0.04	0.19 ± 0.02	–[a]	

[a] Not given.

Ni-IIP cartridges can be used at least forty times without losing the efficiency of the adsorbent IIP for nickel determination; however, for a multi-element pre-concentration, the IIP can only be used twenty-five times.

4. Conclusions

Ion imprinted polymer particles synthesized from a ternary [Ni(II)-DEM-8-HQ] pre-polymer complex have shown imprinting properties for Ni and also for Cu, Pb and Zn. The synthesized polymeric material has not offered affinity for major elements in seawater samples, such as sodium and potassium, efficiently removing the salt matrix of seawater. Therefore, Ni, Cu, Pb and Zn can be measured against external aqueous calibrations in 2.0 M nitric acid. The synthesized IIP can be used at least twenty-five times without losing the adsorbent properties for the target analytes. In addition, the lifetime of the material can be increased up to 40 SPE cycles if only nickel is determined. Because the large life time of the polymer and the low cost of the synthesis, the Ni-IIP results cost effective when comparing to other adsorbents commonly used for SPE. The fast kinetics for adsorption/elution is other advantage. Volumes of 100 mL of seawater can be treated in 40 min. When using a vacuum manifold station and several samples are simultaneously treated, a sampling frequency of nine samples per hour is achieved. Finally, the IIP-SPE–ICP-OES/MS method has offered accurate results for the analysis of low salinity samples (lake water) and high salinity samples (estuarine water).

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ORIGINAL PAPER

On-line ionic imprinted polymer selective solid-phase extraction of nickel and lead from seawater and their determination by inductively coupled plasma-optical emission spectrometry

Natalia García-Otero · Carmen Teijeiro-Valiño · Jacobo Otero-Romaní · Elena Peña-Vázquez · Antonio Moreda-Piñeiro · Pilar Bermejo-Barrera

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Abstract Nickel(II) and lead(II) ionic imprinted 8-hydroxyquinoline polymers were synthesized by a precipitation polymerization technique and were used as selective solid phase extraction supports for the determination of nickel and lead in seawater by flow injection solid phase extraction on-line inductively coupled plasma-optical emission spectrometry. An optimum loading flow rate of 2.25 mL min^{-1} for 2 min and an elution flow rate of 2.25 mL min^{-1} for 1 min gave an enrichment factor of 15 for nickel. However, a low dynamic capacity and/or rate for adsorption and desorption was found for lead ionic imprinted polymer and a flow rate of 3.00 mL min^{-1} for 4-min loading and a flow rate of 2.25 mL min^{-1} for 1-min elution gave a enrichment factor of 5. The limit of detection was 0.33 μg L^{-1} for nickel and 1.88 μg L^{-1} for lead, with a precision (n=11) of 8% (2.37 μg Ni L^{-1}) for nickel and 11% (8.38 μg Pb L^{-1}) for lead. Accuracy was also assessed by analyzing SLEW-3 (estuarine water) and TM-24 (lake water) certified reference materials, and the values determined were in good agreement with the certified concentrations.

Keywords On-line solid-phase extraction · Ionic imprinted polymer · Nickel · Lead · Seawater · Inductively coupled plasma-optical emission spectrometry

N. García-Otero · C. Teijeiro-Valiño · J. Otero-Romaní · E. Peña-Vázquez · A. Moreda-Piñeiro · P. Bermejo-Barrera (✉)
Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n,
15782 Santiago de Compostela, Spain
e-mail: pilar.bermejo@usc.es

Introduction

Nickel and lead have long been recognized to be toxic metals for marine ecosystems. Both elements enter the aquatic environment from the dissolution of rocks and soils, from atmospheric fallout, but mainly from industrial processes and water disposal [1, 2]. This last fact is especially important for estuarine systems because they are near different industries and villages located along the coast. In addition, estuaries are also used for different aquaculture activities, such as mollusk production, and the levels of heavy elements in estuarine water are limited by several legislation directives [3]. Therefore, continuous development of reliable analytical methods to assess these elements in complex samples is of interest.

Heavy metals are present at very low concentrations in estuarine/seawater, whereas matrix concomitants such as chloride and alkaline elements (sodium, potassium, etc.) occur at very high levels [1]. Therefore, preconcentration/separation methods combined with sensitive and selective detectors are needed to perform nickel and lead determination in seawater. Among the preconcentration procedures for trace elements, solid phase extraction (SPE) techniques are quite popular because they offer a number of important advantages over other preconcentration methods. One of the main benefits is that adsorbent material can be packed into minicolumns or cartridges which provide sufficient exchange/adsorption capacity for many applications and ease of automation [4]. Most of the SPE procedures use ion-exchange/chelating resins, which have the disadvantage of a previous derivatization stage when preconcentrating inorganic species such as trace metals. This is because only uncharged (nonpolar) species can interact with most of the

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adsorbent materials [5]. For example, when high-purity C_{18} (silica-based materials) is used as an adsorbent material for lead and/or nickel, a chelating reagent such as 8-hydroxyquinoline (8-HQ) is needed to obtain interactions between uncharged complexes and the adsorbent [6–8]. In addition, another disadvantage of such materials for metal preconcentration is the lack of selectivity, which generates a matrix effect, and the use of the standard addition technique for carrying out the determinations [8].

High selectivity for SPE procedures can be obtained by using solid supports synthesized by molecular imprinting polymerization or by ionic imprinting polymerization. These materials can be prepared (synthesized) using inexpensive reagents and with a general knowledge of synthesis. For either molecular imprinted polymer (MIP) or ionic imprinted polymer (IIP), a monomer is polymerized in the presence of a template (a molecule, an ion, or a metal complex) and an excess of a cross-linker, and after the template leaching, the MIP (IIP) is capable of recognizing and rebinding the target analyte (the template) with high selectivity and affinity [9, 10]. Among the different approaches for the synthesis of IIPs [11, 12], the trapping of a nonvinylated chelating ligand (complexing agent) via imprinting of binary/ternary mixed ligand complexes of metal ions with a nonvinylated chelating agent and a vinyl ligand is a popular method [12]. The complexing agent is trapped in the polymeric material, showing affinity for the ion (template) which was used for synthesis. Complexing agents such as dimethylglyoxime [13], diazoaminobenzene [14], cetyltrimethylammonium bromide [15], and derivates of quinoline (5,7-dichloroquinolin-8-ol) [16–25] have been successfully applied, mainly for lanthanides, although some applications can be found in the literature for transition metals such as nickel [24, 25].

All the IIP-based SPE procedures mentioned above are off-line processes, which are time-consuming. If the IIP materials can offer high dynamic capacity and/or rates for adsorption and desorption, preconcentration can be carried out without reaching the thermodynamic equilibrium in short times, being suitable for on-line preconcentration approaches. Some examples of on-line SPE using ionic imprinted sorbents can be found in the literature for both lanthanides [26] and transition metals such as copper [27]. Therefore, one of the goals of the current work is to evaluate the possibilities of two synthesized IIPs for the on-line preconcentration/separation of traces of nickel and lead from seawater samples. Synthesized IIP adsorbent material was packed into short cartridges which were incorporated in a six-way valve of an automatic flow injection manifold combined with inductively coupled plasma-optical emission spectrometry (ICP-OES) detection. Inexpensive reagents such as 8-HQ, as a nonvinylated reagent, 2-(diethylamino) ethyl methacrylate (DEM), as a monomer, and divinylbenzene-80 (DVB), as a cross-linker, were used for synthesis by the precipitation polymerization technique.

Experimental

Apparatus

An Optima 3300 DV inductively coupled plasma atomic emission spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with an AS 91 autosampler (PerkinElmer) and a Gem-Cone cross-flow nebulizer (PerkinElmer) was used for lead and nickel determinations. The detection wavelengths were 231.604 and 224.688 nm for nickel and lead, respectively. The radiofrequency power was 1,300 W and the argon flow rates were 15.0 $L min^{-1}$ for plasma, 0.5 $L min^{-1}$ for the auxiliary gas, and 0.8 $L min^{-1}$ for the nebulization. A Rheodyne (model 5020) six-port low-pressure flow-injection valve (Rheodyne, Cotati, CA, USA) with polytetrafluoroethylene tubes (0.8-mm inner diameter) was connected to a peristaltic pump (PerkinElmer) for direct introduction of the analyte to the nebulizer-plasma torch system, and to a Miniplus 3 peristaltic pump (Gilson, Middleton, WI, USA) for loading the sample and pumping the eluting solution. Solvent flexible tubing red/red, 1.14 mm (SCP Science, Montreal, Canada) was used for loading the sample and pumping the eluting solution through the cartridges, whereas solvent flexible tubing black/black, 0.76 mm, and white/black, 1.52 mm (SCP Science), was used for introduction of the eluate into the plasma torch and for waste removal, respectively. Synthesized IIP material (300 mg) was placed into empty Sep-Pak® cartridges (plus short) from Waters (Milford, MA, USA) between 20-μm-porosity Teflon frits (Supelco, Bellefonte, PA, USA). A Boxcult incubation camera from Selecta (Barcelona, Spain) with controlled temperature coupled with a low-profile roller (Stovall, Greensboro, NC, USA) was used for the polymerization process. An Orion 720A plus pH meter with a glass-calomel electrode (Orion, Cambridge, UK) was used for pH measurements.

Reagents

Chemicals were ultrapure grade and uultrapure water, resistance 18 MΩ cm (Millipore, Bedford, MA, USA), was used. Stock nickel and lead standard solutions (1.000 gL^{-1}) were supplied by Merck (Darmstadt, Germany). Analytical grade nickel chloride ($NiCl_2.6H_2O$) and lead nitrate ($Pb(NO_3)_2$), used as templates, were purchased from Panreac (Barcelona, Spain). High-purity 69% nitric acid was from Panreac, and high-purity ammonia, ammonium chloride, and analytical grade 8-HQ were purchased from Merck. High

performance liquid chromatography grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). DEM and DVB were from Sigma-Aldrich (Steinheim, Switzerland). DVB was treated to remove the polymerization inhibitor by passing a few milliliters of the reagent through a minicolumn containing approximately 0.5 g of neutral alumina (Sigma-Aldrich). 2,2′-Azobisisobutyronitrile was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at −20 °C after dissolving the reagent in methanol (Merck) at 50–60 °C. After purification, this reagent was stored at 4 °C. Estuarine seawater (SLEW-3) certified reference material was obtained from the National Research Council of Canada. Lake water (TM-24) certified reference material was purchased from the National Water Research Institute of Canada.

Collection of seawater samples

Seawater samples (1 L) were collected from the Ría de Muros-Noia estuary (northwestern Spain) in precleaned high density polyethylene bottles. After collection, seawater samples were filtered through 0.45-μm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then stored at low temperature until they were used.

Synthesis of nickel and lead IIPs

Table 1 lists the amounts (weights or volumes) of template (nickel chloride or lead nitrate), monomer (DEM), complexing agent (8-HQ), cross-linker (DVB), and free radical initiator (2,2′-azobisisobutyronitrile) used in the synthesis of nickel IIP [25] and lead IIP. The general procedure was as follows. First, the template was mixed with the monomer and the complexing agent in 15-mL glass tubes before 12.5 mL of porogen (3:1 acetonitrile/toluene) was added. The mixture was then stirred for 5 min and filtered if necessary. Finally, the cross-linker and the initiator were added to the mixture (volumes or weights given in Table 1) and the glass tubes were purged with N_2 and immediately sealed just before thermal induction of the precipitation polymerization. The temperature was increased from room temperature to 60 °C over 2 h, and then maintained at 60 °C for a further 24 h under a low stirring rate (33 rpm). The polymer particles obtained were then vacuum-filtered, washed with acetonitrile, and oven-dried overnight at 40 °C. It can be seen from Table 1 that the polymers were synthesized in the presence of a double amount of 8-HQ (complexing agent) with respect to DEM (monomer). This gives a template/DEM/8-HQ molar ratio of 1:2:4. The masses of polymer obtained after polymerization were around 0.300 g and the efficiency of the polymerization process, taking into account a theoretical amount of synthesized polymer of 0.5 g, was around 60%. Blank polymer particles (nonimprinted polymer, NIP) were also prepared in the same way as IIP, but without the template. The NIP was then subjected to the same washing pretreatment as described above.

Preparation of IIP cartridges

Empty Sep-Pak® cartridges were filled with 0.30 g of IIP or NIP adsorbents. The IIP or NIP material was between two Teflon frits. Once prepared, the IIP cartridge was incorporated in the six-way valve and the template [nickel(II) or lead(II) ions] was removed from the polymer particles by extensive washing with 2.0 M nitric acid. An efficient removal of the template from IIP particles was achieved after passing 50 mL of the washing solution. This was verified by ICP-OES measurements of nickel or lead in the washing (eluting) solutions from the cartridges (six-way valve in injection position, according to Fig. 1).

On-line preconcentration-ICP-OES determination

Under optimum conditions, the pH of the acidified seawater samples was adjusted to 9.0 for nickel preconcentration or to 8.5 for lead preconcentration by adding low amounts of 6.0 M ammonia solution or by preparing an aqueous standard in 0.1 M NH_3/0.1 M NH_4Cl buffer solution at pH

Table 1 Molar template/monomer/ligand ratio and masses and volumes of the different reagents involved into the polymerization process

	Template (g)	Monomer (DEM) (μL)	Ligand (8-HQ) (g)	Cross-linker (DVB) (μL)	Initiator (AIBN) (g)	Molar ratio (template/monomer/ligand)	Mass of polymer (g)
Ni IIP	0.0334[a]	56.5	0.0820	500	0.0420	1:2:4	0.3121 (62%)
Pb IIP	0.0355[b]	60.0	0.0842	500	0.0413	1:2:4	0.3080 (62%)

DEM (diethylamino) ethyl methacrylate, *8-HQ* 8-hydroxyquinoline, DVB divinylbenzene-80, *AIBN* 2,2′-azobisisobutyronitrile, *IIP* ionic imprinted polymer

[a] $NiCl_2 \cdot 6H_2O$

[b] $Pb(NO_3)_2$

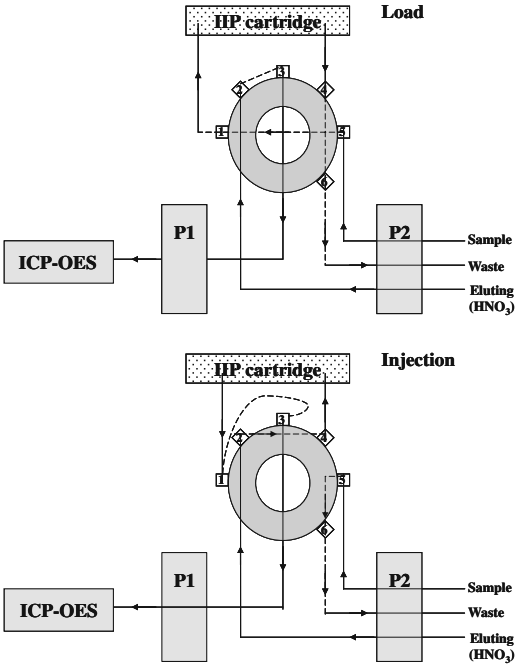

Fig. 1 The flow-injection manifolds used for the preconcentration-inductively coupled plasma-optical emission spectrometry (*ICP-OES*) determination. *IIP* ionic imprinted polymer, *P1* pump 1, *P2* pump 2

9.0 (nickel preconcentration) or 8.5 (lead preconcentration). A similar procedure was adopted when testing other pHs (6.0, 8.0, 8.5, and 9.0) as described in "Effect of pH: imprinting effect." A diagram showing the loading (adsorption) and elution steps in the on-line nickel or lead IIP SPE is given in Fig. 1. In the preconcentration (loading) step, the seawater sample (at pH 9.0 for nickel or at pH 8.5 for lead) is drawn through the cartridge containing the IIP material by means of a flow rate of 2.25 mL min^{-1} for 120 s (nickel preconcentration) or 3.00 mL min^{-1} for 240 s (lead preconcentration). In the meantime, the eluting solution (3.0 M nitric acid) is pumped by pump 2 and pump 1 through the detector (Fig. 1a). In the elution step (Fig. 1b), the eluting solution is propelled through the column in the opposite direction to the loading at a flow rate of 2.25 mL min^{-1} for 60 s, time enough to elute the analytes completely, as is shown in Fig. 2 (FIAgrams for nickel and lead measurements). The eluting solution is directly introduced to the detector and the nickel or lead emission signals are continuously monitored. Previous to the loading step, the IIP material is conditioned by passing a 0.1 M NH_3/0.1 M NH_4Cl buffer solution at pH 9.0 (nickel preconcentration) or 8.5 (lead preconcentration) through the cartridge at a flow rate of 2.25 mL min^{-1} for 60 s (optimum conditions), or by passing 0.1 M NH_3/0.1 M NH_4Cl buffer solution at other pHs for optimization studies (see "Effect of pH: imprinting effect"). Similarly, the same buffer solution at the same flow rate and time conditions was passed through the cartridges just before the elution step to remove adsorbed matrix concomitants from the IIP material.

Determinations were performed by using aqueous standard solutions in 0.1 M NH_3/0.1 M NH_4Cl at pH 9.0 (nickel) or 8.5 (lead) covering nickel concentrations within the 0–50 μg L^{-1} range and lead concentrations until 100 μg L^{-1}.

Results and discussion

Preliminary evaluation of IIPs under static conditions

To measure the static adsorption capacity, 300 mg of imprinted (nickel IIP and lead IIP) sorbents were saturated (flow rate of 10 mL min^{-1}) with several 3.0-mL aliquots of 200 μg mL^{-1} nickel(II) or 100 μg mL^{-1} lead(II) solutions at pH 8.5 [8, 25] and then the nickel or lead content in the eluates was directly measured by ICP-OES. The static adsorption capacities of the nickel IIP and lead IIP were 0.023 and 0.0015 mmol g^{-1}, respectively, which implies calculated retention capacities of 11 and 7% for nickel IIP and lead IIP, respectively. These calculated retention

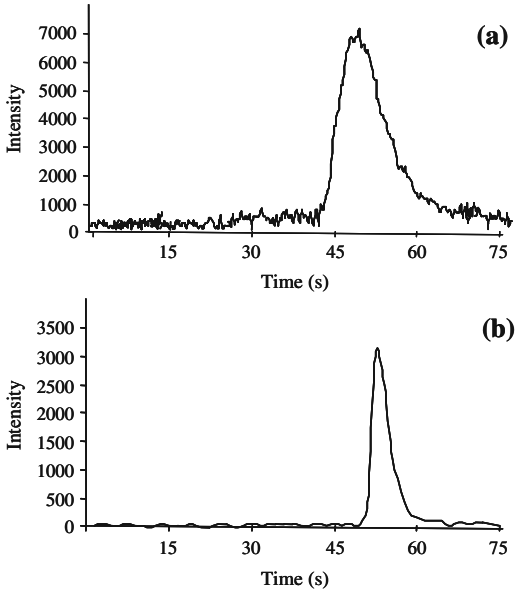

Fig. 2 FIAgram for a seawater sample spiked with 20 μg L^{-1} nickel (II) (**a**) or 20 μg L^{-1} lead(II) (**b**)

capacities are around 10% of the theoretical retention capacity, which agrees with reported retention capacities for MIPs [28].

Similarly, the breakthrough volume was determined under static conditions. Different volumes (25, 100, and 200 mL) of aqueous nickel(II) or lead(II) standard solution, both at 50 μg L^{-1}, were adjusted to pH 8.5 [8, 25], and were passed through cartridges packed with 300 mg of both IIPs at a flow rate of 10 mL min^{-1}. The retained nickel or lead was then eluted with 2.5 mL of 2.0 M nitric acid and determined by ICP-OES. It was observed that large volumes of loading solutions can be used for both IIPs containing 300 mg of the polymeric material and the breakthrough volume is not reached even after loading with 200 mL of sample solutions, showing an enhancement factor of 80 under static conditions.

Optimization of loading conditions (dynamic adsorption conditions)

The loading flow rate and loading time were first studied under fixed elution conditions: an elution flow rate of 3.0 mL min^{-1}, a nitric acid concentration in the eluting solution of 2.0 M, and an elution time of 1.0 min. Aqueous standard solutions containing nickel(II) or lead(II) at 50.0 μg L^{-1} or seawater samples spiked with nickel(II) or lead (II) at 20.0 μg L^{-1} were used as samples. Nickel(II) or lead (II) aqueous standards were prepared in a 0.1 M NH_3/0.1 M NH_4Cl/ buffer solution at pH 8.0. The pH of the spiked seawater samples, acidified with nitric acid added, was increased to 8.0 by adding small volumes of 5.0 M NH_3. This pH was selected on the basis of previous off-line experiments for metal-8-HQ complexes and C_{18} support materials and also for IIPs for Ni(II) preconcentration [8, 25]. A washing stage just before the elution stage was applied by pumping a 0.1 M NH_3/0.1 M NH_4Cl buffer solution at pH 8.0 at a flow rate of 3.0 mL min^{-1} for 1.0 min.

Figure 3 shows the average peak height (three replicates) for spiked seawater samples when varying the loading flow rate from 1.25 to 3.5 mL min^{-1} and for loading times within the 0.5–4.0-min range. It was found that the nickel intensity slowly decreased when the load flow rate was increased from 2.25 to 3.50 mL min^{-1}, whereas high lead signals were obtained for high load flow rates (Fig. 3a). Similarly, the highest nickel signals were obtained for load times above 2.0 min. However, a higher load time is needed to obtain the highest lead signal (Fig. 3b). These results imply a low dynamic capacity and/or rate for adsorption/desorption for lead IIP. A load flow rate of 3.00 mL min^{-1} for a load time of 4.0 min is needed to preconcentrate lead, whereas a load flow rate of 2.25 mL min^{-1} for a load time of 2.0 min is enough for the use of nickel IIP. Similar

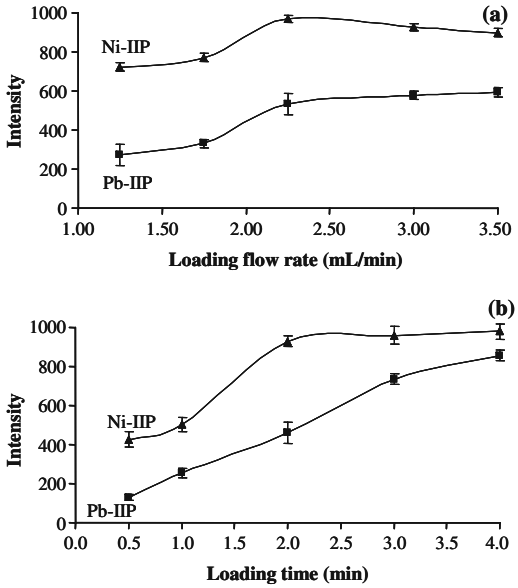

Fig. 3 Effect of the loading flow rate (**a**) and the load time (**b**) on the nickel and lead emission intensities (peak height)

results were obtained when nickel(II) or lead(II) buffered aqueous standard solutions were used. Therefore, under these loading conditions, a volume of 14 mL was passed through the cartridge containing lead IIP but only 4.5 mL was required for nickel IIP.

Optimization of elution conditions (dynamic adsorption conditions)

By fixing the loading conditions at 2.25 mL min^{-1} for 2.0 min (nickel IIP) and at 3.00 mL min^{-1} for 4.0 min (lead IIP), we studied the elution flow rate from 1.25 to 3.5 mL min^{-1}. A 2.0 M nitric acid solution was used as the eluting solvent and also either nickel(II) or lead(II) aqueous standard solutions and spiked seawater samples were buffered at pH 8.0. Figure 4a shows the nickel signal decreases when elution with a flow rate higher than 2.25 mL min^{-1} is used, whereas the lead signal remains constant within the elution flow rate studied.

The effect of the nitric acid concentration on the nickel and lead signals is plotted in Fig. 4b for an elution flow rate of 2.25 mL min^{-1}. It can be seen that a nitric acid concentration of 3.0 M was sufficient to elute both metals from the polymer particles.

Similar results were obtained for the use of nickel(II) or lead(II) buffered aqueous standard solutions. In addition, the time for the washing stage between the loading and

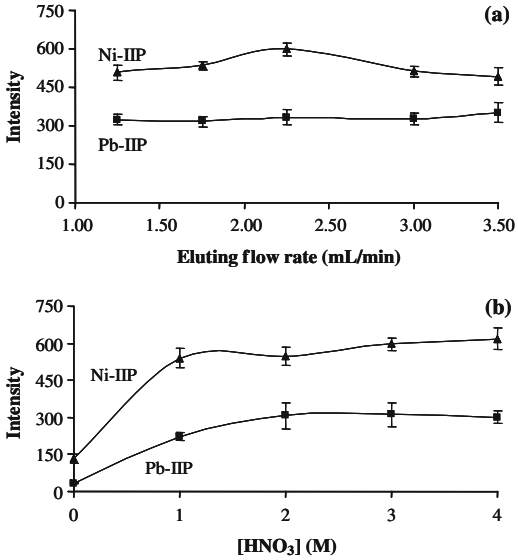

Fig. 4 Effect of the elution flow rate (**a**) and the nitric acid concentration (**b**) on the nickel and lead emission intensities (peak height)

injection steps was not found to be significant, and a washing time of 1.0 min was finally selected.

Effect of pH: imprinting effect

The effect of the pH on the nickel(II) or lead(II) retention was studied for cartridges filled with synthesized nickel IIP and lead IIP materials as well as for nickel and lead NIPs. Different buffered aqueous standards (at the pHs studied) covering nickel or lead concentrations between 0 and 50 μg L^{-1} and an unspiked seawater sample and seawater spiked with 20 μg L^{-1} nickel(II) or lead(II) were used for the experiment. The results as analytical recoveries are shown in Fig. 5, plot a for nickel IIP and nickel NIP, and in Fig. 5, plot b for lead IIP and lead NIP. It can be seen that nickel is quantitatively retained when a pH between 8.0 and 9.0 is used (nickel recoveries from 104±4 to 100±2%), whereas nickel recoveries when the NIP is used are lower than 6% for all pHs. This means that interactions between nickel ions and the polymer particles are specific interactions (imprinting effect) even at pH 6.0 (nonquantitative retention). Therefore, it can be said that imprinted cavities for nickel(II) were produced during the synthesis of nickel IIP.

Quantitative lead recoveries (Fig. 5, plot b) were only reached at pHs of 8.0 and 8.5 (lead recoveries of 99±5 and 103±4%, respectively), giving lead recoveries of around 80% when using pHs of 6.0 and 9.0. However, lead recoveries close to 60% were obtained for the use of lead NIP at pHs of 8.0 and 8.5. This means that interactions between lead ions and the polymer particles can be attributed to specific (imprinting) and nonspecific (adsorption) interactions. Although a high percentage extraction (around 60% in Fig. 5, plot b) was obtained for the use of lead NIP, the lead IIP offers imprinting capabilities for lead (II) ions because the difference between the percentage extraction of the lead IIP and lead NIP is close to 40%. In this sense, other IIPs reported in the literature have produced differences lower than 30% [16, 23] or even lower than 20% [17]. It must be noted that the lead IIP does not work as an imprinted polymer at pH 6.0 since lead recoveries close to 80% for both nickel IIP and lead NIP are obtained.

From the results obtained, a pH of 9.0 was finally chosen for nickel preconcentration, whereas a pH of 8.5 was preferred for lead.

Effect of major components from seawater

A set of experiments was carried out to observe interactions between the polymeric materials and the major metals present in seawater (Na, K, Ca, and Mg). A seawater sample was subjected seven times to the proposed procedure and the concentrations of the major ions were determined by ICP-OES. After preconcentration, concentrations lower than 20 mg L^{-1} were determined for sodium,

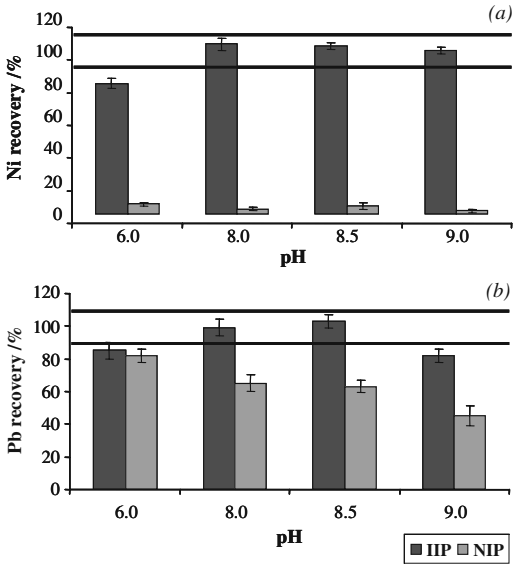

Fig. 5 Effect of pH on the analytical recovery for nickel (*a*) and lead (*b*) when using IIP and nonimprinted polymer (*NIP*)

potassium, magnesium and calcium, which are very low concentration taking into account the concentration of such elements in seawater, around 11,490, 399, 1,293 and 413 mg L^{-1}, for sodium, potassium, magnesium, and calcium, respectively [1]. Therefore, it can be concluded that the salt matrix is efficiently removed for the use of either nickel IIP or lead IIP.

Cross-reactivity among nickel/lead and other trace elements

To evaluate the cross-reactivity among nickel or lead and other transition metals (Cd, Cu, Cr, Fe, Mn, Sn, V, and Zn), aqueous solution containing 50 μg L^{-1} of the target element [nickel(II) or lead(II)] and the other trace metals (also at 50 μg L^{-1}) were passed through the nickel IIP (and nickel NIP), and also the lead IIP (and lead NIP), in triplicate after the pH had been fixed at 9.0 (nickel IIP and nickel NIP) or 8.5 (lead IIP and lead NIP). The results, expressed as percentage extraction, distribution ratios, and selectivity coefficients are listed in Tables 2 and 3 for nickel IIP/NIP and lead IIP/NIP, respectively. From these results (Table 4), it can be stated that lead IIP showed good selectivity for lead over other transition metals, and imprinting properties are only observed for lead. In should be mentioned that chromium is quantitatively retained in lead IIP, but this retention is unspecific (a similar percentage extraction is obtained for chromium when lead NIP is used). However, nickel IIP showed a certain imprinting effect for copper, lead, and zinc, and also nonspecific interactions for chromium (Table 3). The relatively low selectivity of nickel IIP can be attributed to the fact that the hydroxyquinoline ring (8-HQ as a ligand) is not linked to the polymeric chains forming rigid imprinting cavities. In this case, 8-HQ is trapped in the polymeric matrix after polymerization and the imprinting cavities appear to offer certain "flexibility". Because of the multielement capabilities of nickel IIP, this polymer can be used for the simultaneous preconcentration of nickel, copper, lead, and zinc from seawater samples.

Table 2 Percentage extraction (*E*), distribution ratios (*D*), and selectivity coefficients ($S_{Ni/M}$) for nickel IIP and nickel nonimprinted polymer (*NIP*)

Element	IIP			NIP		
	E	*D*	$S_{Ni/M}$	*E*	*D*	$S_{Ni/M}$
Ni	99	99	–	1	0.01	–
Cd	76	3	33	13	0.2	0.05
Cu	93	13	8	39	0.6	0.02
Cr	98	49	2	99	99	0.0001
Fe	82	5	20	84	5	0.002
Mn	20	0.2	495	12	0.1	0.1
Pb	95	19	5	34	0.5	0.02
Sn	45	0.8	124	78	3	0.003
V	90	9	11	75	3	0.003
Zn	90	9	11	37	0.6	0.02

E (%) = $(A_2/A_T) \times 100$, $D = (A_2/A_1)$, and $S_{Ni/M} = D_{Ni}/D_M$, where A_1 is the amount of metal ion in aqueous solution at equilibrium, A_2 is the amount of metal ion enriched by IIP/NIP at equilibrium, A_T is the total amount of metal ion used in extraction, D_{Ni} is the distribution ratio for nickel, and D_M is the distribution ratio for metal M (M is Cd, Cu, Cr, Fe, Mn, Pb, Sn, V, and Zn).

Table 3 Percentage extraction (*E*), distribution ratios (*D*), and selectivity coefficients ($S_{Pb/M}$) for lead IIP and lead NIP

Element	IIP			NIP		
	E	*D*	$S_{Ni/M}$	*E*	*D*	$S_{Ni/M}$
Pb	99	99	–	55	1	–
Cd	76	3	33	22	0.3	3
Cu	80	4	25	76	3	0.3
Cr	92	12	8	95	19	0.05
Fe	85	6	16	81	4	0.2
Mn	75	3	33	70	2	0.5
Ni	85	6	16	75	3	0.3
Sn	83	5	20	80	4	0.2
V	80	4	25	75	3	0.3
Zn	57	1	76	40	0.7	1

E (%) = $(A_2/A_T) \times 100$, $D = (A_2/A_1)$, and $S_{Pb/M} = D_{Pb}/D_M$, where A_1 is the amount of metal ion in aqueous solution at equilibrium, A_2 is the amount of metal ion enriched by IIP/NIP at equilibrium, A_T is the total amount of metal ion used in extraction, D_{Pb} is the distribution ratio for lead, and D_M is the distribution ratio for metal M (M is Cd, Cu, Cr, Fe, Mn, Ni, Sn, V, and Zn).

Analytical performances

Five different buffered aqueous calibrations and standard addition graphs (pH 9.0 for nickel or 8.5 for lead), both covering nickel concentrations from 0 to 50 μg L^{-1} or lead concentrations from 0 to 100 μg L^{-1}, were obtained. Mean slopes of 126.2±9.161 and 109.5±10.73 L μg^{-1} were obtained for nickel aqueous standard and nickel standard addition graphs, respectively, whereas a mean value of 49.1±2.44 L μg^{-1} was obtained for lead aqueous standards and a mean value of 48.2±1.72 L μg^{-1} was obtained for lead standard addition.

After application of Cochran's and Bartlett's tests at the 95.0% significance level to comparing variances and an analysis of variance test for comparison of means, also at the 95.0% significance level, it could be concluded that the slopes of the aqueous calibration and standard addition graphs for nickel and lead are statistically comparable (95.0%) and therefore a matrix effect is not observed for both determinations.

Table 4 Analysis of certified reference materials

	SLEW-3 (estuarine water)		TM-24 (lake water)	
	Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})
Ni	1.23±0.07	1.12±0.08	3.5±3.0	2.5±0.19
Pb	0.0090±0.0014	<1.88	7.3±2.6	6.4±0.38

Each material was analyzed in triplicate.

To obtain the enrichment factor as the ratio of slopes from an aqueous calibration through the cartridges (internal calibration) and from an aqueous calibration without preconcentration (external calibration by direct aspiration of standard solutions), three different aqueous calibrations covering nickel and lead concentrations between 0 and 100 μg L^{-1} were obtained. A mean slope of 8.50±0.955 Lμg^{-1} was calculated for nickel determination, which means an enrichment factor of 14.8. Similarly, a mean slope of 9.50± 1.23 Lμg^{-1} was obtained for the lead external aqueous calibration, meaning an enrichment factor of 5.

The limit of detection (3SD/*m*) and the limit of quantification (10SD/*m*), where SD is the standard deviation of 11 measurements of a blank and *m* is the mean slope of the aqueous calibration graph, were 0.33 and 1.10 μg L^{-1}, respectively, for nickel. Values of 1.88 and 6.27 μg L^{-1} for the limit of detection and the limit of quantification, respectively, were obtained for lead.

The repeatability of the overall procedure was assessed by analyzing a surface seawater sample 11 times by the on-line IIP-SPE-ICP-OES method. The relative SD was 8% for nickel determination (nickel concentration of 2.37±0.181 μg L^{-1}) and 11% for lead (lead concentration of 8.38± 0.907 μg L^{-1}).

The accuracy of the method was assessed by analyzing two certified reference materials: TM-24 (lake water) and SLEW-3 (estuarine water). Each certified reference material was subjected three times to the optimized on-line preconcentration-ICP-OES procedure. Table 4 lists the nickel and lead concentrations found in both certified reference materials. It can be seen that the nickel and lead levels were within the certified concentration ranges, although the results appear to show a small negative bias because both the lead and the nickel values found are on the lower side of the certification range.

Study of the lifetime of the nickel IIP and lead IIP SPE supports

To determine the lifetime of both IIPs, two nickel IIP and two lead IIP cartridges were tested to find out the number of sequential loading/elution cycles that can be performed without loss of preconcentration efficiency. Experiments were carried out by treating estuarine seawater samples (five different SPE cycles) followed by an aqueous standard solution containing 50 μg L^{-1} nickel or 50 μg L^{-1} lead. Analytical recovery was assessed after preconcentrating the nickel or lead aqueous standard solution. The results show that nickel is efficiently retained (nickel IIP can be used at least 40 times; analytical recoveries higher than 95%). For the following ten SPE cycles, the nickel analytical recovery decreased (lower than 95%). However, quantitative analytical recoveries were obtained for lead up to 50 SPE cycles when using lead IIP. Therefore, both IIPs can be used at least 40 times without loss of retention efficiency.

Conclusions

A simple and cheap procedure for the synthesis of IIPs for nickel(II) and lead(II) ions based on trapping a complexing agent (8-HQ) in the polymeric matrix was developed by the precipitation polymerization technique. The IIP materials offer selectivity (imprinting effect) for the template ions [nickel(II) or lead(II)] and they allow a successful separation of these ions from the concomitant seawater matrix components. The synthesized IIP for nickel exhibits better imprinting properties as well as a fast adsorption and desorption rate, which implies an enrichment factor of 15. However, the synthesized IIP for lead exhibits worse imprinting characteristics than those offered by nickel IIP, and an enrichment factor of 5 is achieved for lead. Since the complexing agent is trapped in the polymeric matrix, a previous derivatization stage by the metal complexation is not needed and only a pH adjustment is required before preconcentration. In addition, the synthesized IIPs can be used at least 40 times without losing the adsorbent properties for the target analytes. Because of the long lifetime of the polymer and the low cost for synthesizing it, the nickel IIP is cost-effective compared with other adsorbents for SPE of trace metals. The incorporation of a cartridge filled with the IIP materials in a six-way valve of a flow-injection manifold allows an automated preconcentration and determination of nickel and lead in seawater by ICP-OES. Preconcentration and measurement cycles of 5.0 and 7.0 min were obtained for nickel and lead, respectively,

which implies the analysis of 12 (nickel determinations) or eight (lead determinations) samples per hour. These times are as short as those offered by conventional off-line preconcentration SPE procedures.

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Ionic imprinted polymer for nickel recognition by using the bi-functionalized 5-vinyl-8-hydroxyquinoline as a monomer: Application as a new solid phase extraction support

Jacobo Otero-Romaní [a], Antonio Moreda-Piñeiro [a], Pilar Bermejo-Barrera [a,*], Antonio Martin-Esteban [b]

[a] Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n. 15782 Santiago de Compostela, Spain
[b] Department of Environment, National Institute for Agriculture and Food Research (INIA), Carretera de A Coruña, Km 7.5, 28040 Madrid, Spain

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ABSTRACT

A new ionic imprinted polymer (IIP) for Ni(II) recognition/pre-concentration was prepared via precipitation polymerization using 2-(diethylamino) ethyl methacrylate (DEM) and divinylbenzene (DVB) as a cross-linking agent in the presence of nickel(II) and 5-vynil-8-hydroxyquinoline (5-VHQ) as a bi-functionalized ligand. An important increase on the selectivity of the synthesised IIP for nickel(II) ions was obtained when comparing to the use of 8-hydroxyquinoline (8-HQ) as a ligand. The synthesised IIP was used as a new support for solid phase extraction (SPE) of nickel(II) from seawater before inductively coupled plasma optical emission spectrometry (ICP-OES) detection. Variables affecting the SPE process, such as pH, load and elution flow rates, and concentration and volume of the eluting solution, were fully evaluated. The optimised procedure consists of a sample loading (100 mL of seawater at a pH of 9.0 ± 0.1) through IIP-SPE cartridges containing 300 mg of the synthesised IIP at a flow rate of 3.0 mL min^{-1}. Elution was performed by passing 2.5 mL of 2.0 M nitric acid at a flow rate of 1.5 mL min^{-1}, which gave a pre-concentration factor of 40. The limit of detection (LOD) of the method was 0.26 μg L^{-1}, while the relative standard deviation (RSD) for eleven replicated measurements was 3%. Accuracy of the method was assessed by analyzing SLEW-3 (estuarine water) and TM-23.3 (lake water) certified reference materials. In addition to the selectivity of the synthesised material for nickel(II) ions against other transition metal ions and major alkaline and alkaline-earth metals (Na^+, K^+, Mg^{2+} and Ca^{2+}) in seawater, it can be stated that the salt matrix is efficiently removed by using the proposed IIP-SPE procedure.

1. Introduction

Solid phase extraction is one of the most used techniques for isolating trace elements from liquid samples before analytical determinations mainly by atomic spectrometric methods. However, the main disadvantage of conventional SPE sorbents for trace elements, such as C18, ion-exchange resins and size-exclusion phases, is the lack of selectivity, leading to the co-extraction of matrix concomitants with the target analytes [1]. This fact is specially important when coping with seawater samples because of the high salt content which is an important source of spectral and matrix interferences [2–4]. However, specific SPE sorbents, as immunosorbents (ISs) and molecularly imprinted polymers (MIPs), can avoid this problem by providing selective extraction of such analytes [5].

Synthetic MIPs offer artificial recognition sites which are able to specifically rebind a target molecule in the presence of other similar compounds. These materials can be easily prepared by polymerization of functional and crosslinking monomers around a template molecule (target). As result, a highly cross-linked three-dimensional network polymer is obtained. After polymerization, the template molecule is leached, and the polymer offers binding sites with shape, size and functionalities complementary to the target analyte [6]. Most of the MIPs are prepared by bulk polymerization. After this polymerization process, the material obtained has to be ground and sieved to obtain certain uniformity in particle size. These stages commonly lead to loses of fine particles of the synthesised material. In addition, the particles obtained are normally irregular in size and shape, and some binding sites are partially destroyed during grinding which leads to a considerable loss of loading capacity of the imprinted polymer [7]. To overcome these problems, different polymerization methods (suspension, emulsion, dispersion and precipitation) have been proposed for synthesising MIPs [8]. These synthesis techniques have emerged as appealing methods for producing high-quality imprinted products [9–11], because crushing and sieving steps are avoided and higher yields of reaction are obtained.

IIPs are similar to MIPs but they recognize metal ions after imprinting. For the last years different IIPs have been synthesised for

* Corresponding author. Tel.: +34 600942346.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).

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recognizing lanthanides [12–17], actinides [18–22], noble metals [23,24] and transition elements such as copper [25–27], cadmium [25–30], selenium [31], nickel [32–34], aluminium [35,36], zinc [37], lead [38], iron [39,40], chromium [41] and mercury [42]. Different approaches for the synthesis of IIPs have been reviewed by Rao et al. [43], and four mechanisms can be established: (i) crosslinking of bifunctional reagents with linear chain polymers, (ii) chemical immobilization of vinylated ligands in the polymer matrix, (iii) surface imprinting by emulsion polymerization, and (iv) trapping of a non-vinylated chelating agent inside the polymer matrix. The first three approaches use vinylated reagents which form complexes with the template (metal ion) and can polymerise through the vinyl groups. In such cases, the complexing ligand is chemically immobilized in the polymeric matrix [25,28,29,31,32,36,39]. However, the use of the trapping technique implies that the ligand (a non vinylated reagent) is not chemically bonded to the polymer chains, but it is still trapped inside the polymeric matrix. Different non-vinylated reagents have been successfully applied for synthesising IIPs by using the trapping approach [13,14,16,19–21,23,24,42]. However, low selectivity has been reported for synthesised IIPs based on 8-HQ prepared for recognition of Ni [44]. In this case, the IIP offered recognition capabilities for other transition metals, although the shape and size of such ions was different to those offered by the template [44]. This fact could be explained by keeping in mind that the complexing agent is trapped into the polymeric matrix, but is not linked to the polymer chains, so the imprinting cavities can show certain "flexibility", offering specific interactions between their functional groups and other trace elements rather than the template.

The objective of the current work has been the synthesis of a new IIP for Ni recognition/preconcentration by using 5-VHQ instead of 8-HQ, to fix the complexing agent to the polymeric matrix, and so improving the selectivity. This vinylated reagent was first used by Buono et al. [45,46] for obtaining poly-5-vynil-8-hydroxiquinoline (PVO) as a polymeric material for precipitating trace metals from saline waters [45]; and also for preparing chelating columns by coating on controlled pore diameter glass beads [46]. However, low selectivity was reported for these early applications [45,46]. In the current study, a selective polymeric material based on ionic imprinting methodology was prepared by co-polymerising 5-VHQ with DEM and DVB. The procedure leads to a solid support which is able to selectively rebind nickel(II) in the presence of other trace elements in complex matrices such as seawater.

2. Experimental section

2.1. Instrumentation

Determinations were performed by using an Optima 3300 DV inductively coupled plasma atomic emission spectrometer (Perkin Elmer, Norwalk, CT, USA) equipped with an auto sampler AS 91 (Perkin Elmer) and a Gem-Cone cross-flow nebulizer (Perkin Elmer). The polymerization process was carried out on a temperature-controllable incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA). IIPs were packed into 5 mL cartridges (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA) and an 8-way Gilson peristaltic pump (Gilson, Villiers, France) equipped with 3.18 mm i.d. Tygon tubes (Gilson) was used to drive samples through the IIP cartridges. An ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements. Scanning electron microscope LEO-435VP from LEICA Microsystems (Cambridge, UK), energy dispersive X-ray fluorescence spectrometer (laboratory-made by RIAIDT, University of Santiago de Compostela), EA 1108 element analyser (Fisons, Ipswich, UK) and EA 1108 element analyser (Carlo Erba, Milan, Italy) were used for IIP characterization.

2.2. Reagents

Ultra-pure water of resistivity of 18 MΩcm obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA) was used to prepare the solutions. Analytical grade $NiCl_2 \cdot 6H_2O$ and 69% nitric acid were obtained from Panreac (Barcelona, Spain). Ammonia 25% (v/v) and ammonium chloride were purchased from Merck (Darmstadt, Germany). Single standard solutions (1000 mg L^{-1}) of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V and Zn were also from Merck. 2-(diethylamino) ethyl methacrylate (DEM) was from Sigma-Aldrich (Steinheim, Switzerland). HPLC grade acetonitrile and toluene were purchased from Scharlab (Barcelona, Spain). 5-vynil-8-hydroxyquinoline (5-VHQ) was synthesised at the "Unidad de Química Combinatoria" at the University of Barcelona (Barcelona, Spain). RMN spectra of 5-VQH is given in Fig. S1 (Supplementary data). Divinylbenzene-80 (DVB) from Sigma-Aldrich was treated in order to remove the polymerization inhibitor by passing a few millilitres of the reagent through a mini-column containing 0.5 g of neutral alumina (Sigma-Aldrich). 2,2´-azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at −20 °C after dissolving the reagent in methanol (Merck) at 50–60 °C. After purification, this reagent was stored at 4 °C. Lake water (TM-23.3) certified reference material was purchased from the National Water Research Institute of Canada. Estuarine seawater (SLEW-3) certified reference material was obtained from the National Research Council of Canada.

All glass and plastic materials were rigorously cleaned and kept into 10% (v/v) nitric acid for 48 h. The materials were then rinsed three times with ultra-pure water before being used.

2.3. Seawater collection

Seawater samples (1 L) were collected from the Ría de Muros-Noia estuary (north-western Spain) in pre-cleaned high density polyethylene bottles. After collection, the seawater samples were filtered through 0.45 μm polycarbonate membrane Nucleopore filters (Millipore) and then acidified at a pH lower than 2.0 by adding 1.0 mL of concentrated nitric acid in order to avoid metal adsorption onto the inner bottle walls. Acidified seawater samples were then stored at low temperature until used.

2.4. ICP-OES measurements

Determinations were performed by ICP-OES (axial configuration) using the operating conditions and emission wavelengths given in Table 1. Aqueous standard solutions were prepared in 2.0 M nitric acid covering metal concentrations within 0–4 mg L^{-1} for the optimization process and 0–0.25 mg L^{-1} for nickel IIP-SPE analytical performance studies.

2.5. Synthesis of nickel ionic imprinted polymer particles

The synthesis of IIP particles was carried out by mixing 0.076 g of $NiCl_2 \cdot 6H_2O$, 0.13 mL of DEM and 0.054 g of 5-VHQ in cleaned glass test tubes. Then, 25 mL of porogen (3:1 acetonitrile:toluene) were added, and the solution was stirred for 5 min and then filtrated. Finally, 1.13 mL of DVB (cross-linker) and 0.150 g of AIBN (initiator) were added, the glass tubes were purged with N_2 and immediately sealed just before thermal induction of the precipitation polymerization by increasing the temperature from room temperature to 60 °C over 2 h. The temperature was then maintained at 60 °C whereas a low stirring rate of 33 rpm was used. After 24 h, the polymer was vacuum filtered, washed with acetonitrile and oven-dried at 40 °C. The mass of polymer obtained after polymerization was 0.530 g and the efficiency of the polymerization process, considering 1 g as the theoretical amount of synthesised polymer, was 53%. Non imprinted

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Table 1
ICP-OES operating conditions.

General	
Radio frequency power (W)	1300
Peristaltic pump speed (mL min^{-1})	1.5
Stabilization delay (s)	45
Number of replicates	4
Nebulizer type	Cross flow
Axial view	
Gas flows (L min^{-1})	
Plasma	15.0
Auxiliary	0.5
Nebulizer	0.8
Detection wavelengths (nm)	
Al	396.153
As	228.812
Cd	228.802
Co	238.892
Cr	267.716
Cu	327.393
Fe	238.204
Mn	257.610
Ni	231.604
Pb	224.688
Sn	235.485
Ti	336.121
V	290.880
Zn	213.857

polymer particles (NIP) were also prepared in the same way than IIP, but without the template.

2.6. IIP cartridge packing and template removal procedure

Around 300 mg of the IIP were packed into a 5 mL cartridge between Teflon frits. Afterwards, nickel(II) ions (template) were removed from the polymer particles by extensive washing with 50 mL of 2.0 M nitric acid. Similarly, NIP (300 mg) was also packed and subjected to the same washing pre-treatment.

2.7. IIP solid phase extraction procedure

Aqueous standard solutions (50 μg L^{-1}) were prepared in 100 mL of 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at pH 9.0 ± 0.1. Similarly, 100 mL of acidified seawater samples were treated with 5.0 M ammonia solution (approx. 1 mL), to readjust the pH to 9.0 ± 0.1. The solutions were passed through cleaned and conditioned IIP cartridges at a 3.0 mL min^{-1} flow rate by using a peristaltic pump. The cartridges were then rinsed with 2.5 mL of the 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at pH 9.0 ± 0.1, and then, the retained ions were subsequently eluted with two 1.25 mL aliquots of 2.0 M nitric acid solution at 1.5 mL min^{-1}. A pre-concentration factor of 40 was achieved under these operating conditions. After elution, the IIPs were treated with 10 mL of Milli-Q water and then conditioned by passing 10 mL of the 0.1 M/0.1 M NH_4Cl/NH_3 buffer solution at the working pH (9.0).

3. Results and discussion

3.1. Characterization studies

3.1.1. Scanning electron microscopy

Scanning electron microscopy (SEM) pictures were taken from IIP and NIP (Fig. 1) in order to study the morphology and the size of the materials synthesised. It can be observed that IIP consisted of agglomerates whereas NIP consisted of agglomerates and also spherical monodisperse particles of around 10 μm in diameter. The slightly different polymer morphology can be attributed to the presence of the template in the polymerization process.

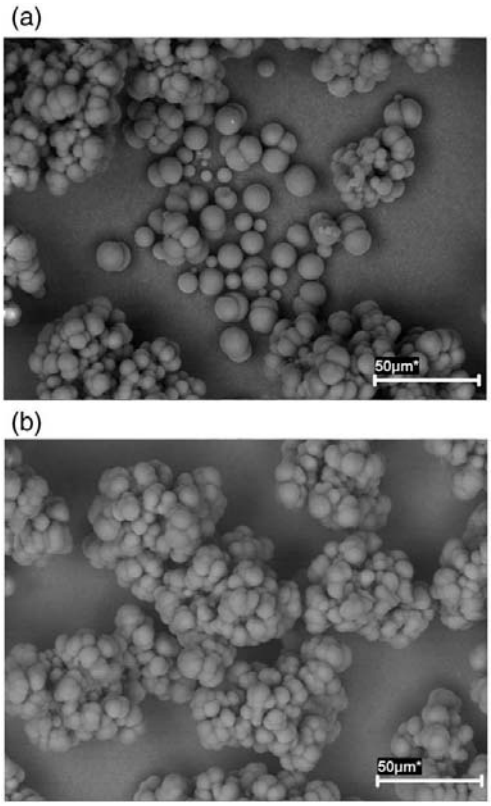

Fig. 1. (a) Scanning electron microscopy picture for NIP. (b) Scanning electron microscopy picture for IIP.

3.1.2. Energy dispersive X-ray fluorescence studies

EDXRF patterns for IIP material before and after leaching, as well as for the corresponding NIP were obtained (Fig. 2). It can be observed that nickel was only present in the unleached IIP particles, and it was totally removed after the template removal procedure.

3.1.3. Microanalysis studies

The elemental (H, C, N and O) composition of IIP and NIP are summarised in Table 2. There is a good agreement between calculated and experimentally found values of H, C, N and O confirming the incorporation of 5-VHQ to the polymeric network.

3.2. Optimization of nickel IIP-SPE from seawater

3.2.1. Effect of pH

A set of experiments was carried out (by triplicate) to determine the optimum pH for nickel SPE. The pre-concentration procedure (Section 2.7) was applied to 100 mL of an aqueous solution containing 50 μg L^{-1} of Ni(II) at four different pHs (from 4.0 to 9.0) fixed by using 0.1 M/0.1 M NH_4Cl/NH_3 buffer solutions. The same experiment was carried out by using the NIP.

Fig. 3 shows the effect of the pH on the analytical recovery of Ni after IIP/NIP-SPE. It can be observed that Ni(II) is quantitatively retained in the IIP at pH 9.0, whereas the analytical recoveries for the non-imprinted polymer are quite lower for all the tested pHs than when using the IIP.

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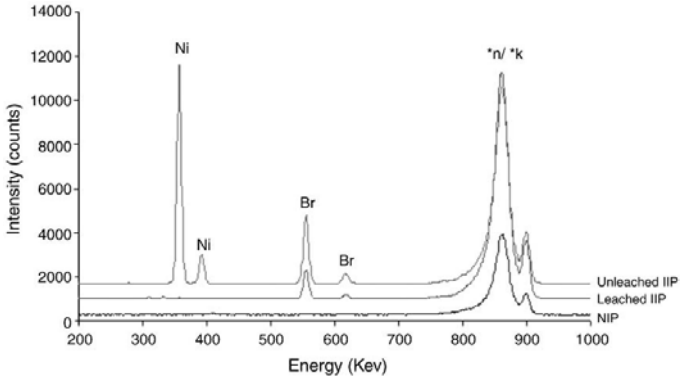

Fig. 2. Energy dispersive X-ray fluorescence spectra for the unleached IIP, leached IIP and NIP.

3.2.2. Effect of the load and elution flow rate

Different experiments were performed in order to find the optimum load and elution flow rates. 100 mL aliquots of an aqueous solution containing 50 μg L^{-1} of Ni(II) were subsequently passed through IIP cartridges at 3.0, 6.0, 9.0 and 12.0 mL min^{-1}. Elution was fixed at 1.5 ml min^{-1} using a 2.0 M nitric acid solution. Results have shown that Ni analytical recovery decreases for load flow rates larger than 3.0 mL min^{-1}.

Similarly, 100 mL aliquots of an aqueous solution containing 50 μg L^{-1} of Ni(II) were passed at 3.0 mL min^{-1}, and eluted with a 2.0 M nitric acid solution at 1.5, 3.0, 4.5 and 6.0 mL min^{-1}. Results have shown that Ni analytical recoveries decrease for elution flow rates larger than 1.5 mL min^{-1}.

In order to achieve the highest nickel analytical recovery, a 3.0 mL min^{-1} load flow rate, and a 1.5 mL min^{-1} elution flow rate were chosen and used for further experiments.

3.2.3. Effect of the nitric acid volume and concentration for elution

Different experiments were performed (by triplicate) in order to find the optimum nitric acid concentration and volume for the eluting solution. After sample loading and rinsing (Section 2.7), the retained analytes were eluted by passing volumes of 2.5 mL of nitric acid at different concentrations (from 2.0 M to 5.0 M). After measuring by ICP-OES against an aqueous calibration prepared at the same nitric acid concentration, similar analytical recoveries were obtained for all cases; thus, an eluting solution of 2.0 M nitric acid was selected for further studies.

Finally, volumes of 2.5, 5.0, 7.5 and 10 mL of a 2.0 M nitric acid solution were tested and quantitative nickel analytical recoveries were reached under all nitric acid volumes. In order to achieve the highest pre-concentration factor, an eluting volume of 2.5 mL (pre-concentration factor of 40) was chosen. It should be mentioned that the elution process must be done in two steps to achieve the highest analytical recovery. Therefore, two 2.0 M nitric acid aliquots of 1.25 mL each were subsequently passed through the IIP cartridges.

3.2.4. Retention capacity

300 mg of polymer were saturated with nickel(II) ions under optimum conditions to determine the retention capacity of the polymer (maximum amount of nickel(II) ions retained per gram of IIP). Several 3.0 mL aliquots of a 200 μg mL^{-1} Ni(II) solution were passed subsequently through the polymer and the nickel content in the eluates was measured by ICP-OES. The retention capacity of the polymer was calculated to be 1.98 mg g^{-1}. The theoretical retention capacity is 18.66 mg g^{-1}; therefore, the calculated retention capacity is around 11% of the theoretical retention capacity. This result agrees with reported retention capacities for MIPs, approximately 10% of the theoretical capacity [47].

3.2.5. Breakthrough volume

Different volumes (from 100 to 500 mL) of an aqueous solution containing 50 μg L^{-1} of nickel(II) were passed through the cartridges (300 mg) at optimum parameters (Section 2.7). All experiments were performed by triplicate and the nickel analytical recoveries found are listed in Table 3.

It can be observed that sample volumes up to 300 mL can be passed through cartridges packed with 300 mg of the polymer without appreciable losses of analytical recovery. This offers a pre-concentration factor of 120 when eluting with 2.5 mL of the eluting solution. However, in order to achieve a reasonable pre-concentration factor in a short time, a sample volume of 100 mL was chosen, so one sample can be pre-concentrated in approximately 35 min with a pre-concentration factor of 40. Therefore, eight samples can be simultaneously pre-concentrated in 35 min by using 8 polymer cartridges and an 8-way peristaltic pump.

3.2.6. Cross-reactivity among nickel and other trace elements

To evaluate the cross-reactivity among nickel and other selected trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Pb, Sn, Ti, V, and Zn) 100 mL of an aqueous solution containing 50 μg L^{-1} of nickel(II) and the other trace metals were passed through the IIP and the NIP (by

Table 2
Elemental composition of IIP and NIP.

	N		C		H		O	
	Calculated (%)	Found (%)	Calculated (%)	Found (%)	Calculated (%)	Found (%)	Calculated (%)	Found (%)
Unleached IIP	1.66	1.38	86.10	85.45	7.66	8.13	2.84	2.51
Leached IIP	1.69	1.77	87.63	86.76	7.79	8.08	2.89	2.48
NIP	1.69	1.40	87.63	88.59	7.79	8.38	2.89	1.72

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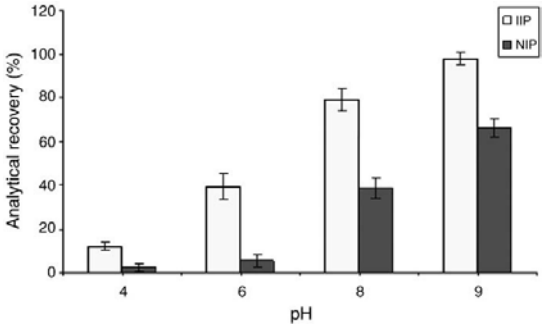

Fig. 3. Effect of the pH on the nickel analytical recovery for IIP and NIP.

triplicate) after fixing the pH at 9.0. Results, expressed as percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$), are listed in Table 4. From these results, the following observations can be made:

1) The IIP material has shown good selectivity for nickel over other transition metals, including Cu, Pb and Zn. As demonstrated in previous works [44], Ni-based IIP synthesised by the same precipitation polymerization technique but using a non-vinylated chelating agent (8-HQ) instead of a vynilated chelating ligand (5-VHQ), offered imprinting properties for the template (nickel (II)) and also for copper(II), lead(II) and zinc(II) with quantitative analytical recoveries. By using 5-VHQ as a bi-functionalised ligand, active groups of the hydroxyquinoline ring are linked to the polymer chains forming rigid imprinting cavities. In this manner, the "flexibility" of the imprinting cavities offered by IIPs based on 8-HQ [44] is avoided, and selectivity for the target ion is highly enhanced.
2) The NIP particles have shown no selectivity for nickel(II) ions over other selected transition metals.
3) The highest selectivity was obtained for the Ni/Al pair, which indicates the two elements having the most important differences in terms of coordination with 5-VHQ and DEM.
4) Although IIP has not shown imprinting properties for Cu, Pb, Zn, Cr, Fe, Sn, Co and Ti, there are some unspecific interactions between these transition metals and the polymeric material with analytical recoveries close to 75%.

3.2.7. Effect of major components from seawater

In order to study the interactions between the polymeric material and the major metals present in seawater (Na^+, K^+, Ca^{2+} and Mg^{2+}), 100 mL of a seawater sample was subjected eleven times to the proposed procedure (Section 2.7) and the concentrations of these elements were determined in the eluates by ICP-OES. After pre-concentration, values of around 24, 2, 22 and 6 mg L^{-1} were found for Na, K, Mg and Ca, respectively. Since the concentrations of Na, K, Mg and Ca in seawater are around 11490, 399, 1293 and 413 mg L^{-1}, respectively [48], it can be concluded that there is not interaction between the polymer particles and the major elements present in seawater.

Table 3
Breakthrough volume.

Sample volume (mL)	Ni analytical recovery (%)[a,b]
100	100 ± 3
200	104 ± 3
300	100 ± 4
400	91 ± 3
500	83 ± 5

[a] $n = 3$.
[b] IIP mass, 300 mg.

Table 4
Percent extraction (E %), distribution ratios (D) and selectivity coefficients ($S_{Ni/M}$) for IIP and NIP.

	IIP			NIP		
Element	E (%)[a]	D[b]	$S_{Ni/M}$[c]	E (%)[a]	D[b]	$S_{Ni/M}$[c]
Ni	98.5	65.7	–	73.2	2.73	–
Cu	74.9	3.0	22.01	76.0	3.17	0.86
Pb	76.2	3.2	20.51	75.0	3.00	0.91
Zn	70.8	2.4	27.08	74.4	2.91	0.94
As	80.6	4.2	15.81	48.9	0.96	2.85
Al	11.6	0.1	500.43	11.1	0.12	21.88
Cd	80.1	4.0	16.31	48.6	0.95	2.89
Cr	79.5	3.9	16.93	83.9	5.21	0.52
Fe	84.8	5.6	11.77	85.0	5.67	0.48
Mn	69.6	2.3	28.68	15.1	0.18	15.36
V	65.4	1.9	34.74	53.2	1.14	2.40
Sn	82.3	4.6	14.12	85.0	5.67	0.48
Co	62.6	1.7	39.23	59.5	1.47	1.86
Ti	79.3	3.8	17.14	83.1	4.92	0.56

A_1 = Amount of metal ion in aqueous solution at equilibrium; A_2 = Amount of metal ion enriched by IIP/NIP at equilibrium. A_T = Total amount of metal ion used in extraction. D_{Ni} = Distribution ratio for Ni; D_M = Distribution ratio for M (M = Cu, Pb, Zn, As, Al, Cd, Cr, Fe, Mn, V, Sn, Co and Ti).
[a] $E(\%) = (A_2/A_T) \times 100$.
[b] $D = (A_2/A_1)$.
[c] $S_{Ni/M} = D_{Ni}/D_M$.

3.3. Analytical performance for nickel determination in seawater by IIP-SPE and ICP-OES

3.3.1. Calibration—evaluation of matrix effect

A comparison between calibration in 2.0 M nitric acid and a standard addition graph was established in order to study possible matrix effects. The standard addition graph was obtained after spiking a pool of eluates (obtained after the pre-concentration of four aliquots of the same seawater sample) with different nickel concentrations (0, 40, 80 and 160 μg L^{-1}). The mean and the standard deviation for the slopes of three standard addition graphs (7.16 ± 0.09 μg L^{-1}) and three external 2.0 M nitric acid calibrations (7.41 ± 0.18 μg L^{-1}) were statistically compared by using the Cochran'C and Bartlett's tests at a 95.0% (comparison of variances), and the ANOVA test (comparison of means). Slopes for external 2.0 M nitric acid calibration and standard addition graphs were statistically similar, so the salt matrix was efficiently removed during the pre-concentration stage. This result agrees with the fact that there is no interaction between major ions present in seawater and the polymeric material (Section 3.6). Therefore, a simple calibration with nickel standard solution in 2.0 M nitric acid is enough to perform seawater analysis for nickel, avoiding the performance of a standard addition graph.

3.3.2. Sensitivity of the method

The limits of detection and quantification were calculated according to LOD = (3*SD)/m and LOQ = (10*SD)/m, where SD is the standard deviation of eleven measurements of a procedural blank (acidified Milli-Q water treated as a sample) and m is the slope of an external 2.0 M nitric acid calibration graph. The limit of detection was calculated to be 0.26 μg L^{-1} for a pre-concentration factor of 40.

Table 5
Analysis of certified reference materials ($n = 2$).

SLEW-3		TM-23.3	
Certified value (μg L^{-1})	Found value (μg L^{-1})	Certified value (μg L^{-1})	Found value (μg L^{-1})
1.23 ± 0.07	1.17 ± 0.31	5.4 ± 1.1	6.1 ± 0.4

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Similarly, the limit of quantification was calculated to be 0.87 μg L^{-1}. Such LODs and LOQs are low enough to determine nickel in unpolluted seawater samples. These values are similar to those reported by other authors when using IIPs combined with ETAAS (0.3 μg L^{-1} [32] or 0.18 μg L^{-1} [34]) or ICP-OES (0.16 μg L^{-1} for [33]) detection.

3.3.3. Repeatability and accuracy of the method

To assess the repeatability of the overall procedure (IIP-SPE and ICP-OES determination) a seawater sample was analysed eleven times by the proposed procedure (Section 2.7), giving a mean nickel concentration of 0.93 μg L^{-1} and a R.S.D of 3%, which confirms good repeatability of the overall procedure.

The accuracy of the method was verified by studying the analytical recovery and by analyzing two certified reference materials with different salinities (SLEW-3 and TM-23.3). Analytical recovery was assessed for three nickel concentration levels, after spiking three different aliquots from the same seawater sample with 1.0, 2.0 and 4.0 μg L^{-1} of Ni. Each nickel concentration was performed by triplicate. The analytical recoveries obtained were 99 ± 3%, 98 ± 3% and 99 ± 3% for 1.0, 2.0 and 4.0 μg L^{-1} of Ni, respectively, so it can be concluded that complete analytical recovery was obtained for all the nickel concentration levels studied. SLEW-3 (estuarine water) and TM-23.3 (lake water) certified reference materials were analysed by duplicate by using a sample volume of 100 mL (pre-concentration factor of 40). Results, listed in Table 5, reveal good agreement between found concentrations and certified values for both certified reference materials. This fact has been verified after applying the *t*-test for means comparison.

3.3.4. Study of the lifetime of the Ni-IIP support

Different Ni-IIP cartridges were tested in order to know the number of sequential loading/elution cycles that can be performed without losses on pre-concentration efficiency. These experiments were carried out by treating aqueous standard solutions containing 1.0 μg L^{-1} of Ni (II), and calculating the analytical recovery after ICP-OES measurement. Before passing each Ni(II) aqueous standard solution, the same cartridges containing the IIP, were previously used five times for pre-concentrating different estuarine water samples. It has been found that quantitative nickel recoveries (higher than 95%) were reached within the fifty loading/elution cycles, and analytical recoveries lower than 95% were obtained after fifty SPE cycles. Therefore, IIP cartridges can be used at least fifty times without losing of efficiency of the adsorbent IIP for nickel determination.

4. Conclusions

The synthesised Ni based IIP by using a vynilated bi-functionalised reagent (5-VHQ) offers higher selectivity for the target ion (nickel(II)) than that offered by Ni-based IIPs synthesised in the presence of 8-HQ [44]. This result is attributed to the fact that the vynilated ligand is linked to the polymer chains rather than being trapped into the polymeric matrix. Therefore, rigid imprinting cavities are obtained and high nickel(II) ion recognition capabilities are achieved. These results suggest that a low-selective complexing agent without vynil groups, such as 8-HQ, can be transformed into a selective agent by adding a vynil group and subjecting it to an ionic-imprinting polymerization process. In addition to selectivity over other transition metals, the new IIP material has not offered affinity for major elements in seawater, such as sodium and potassium, allowing the determination of Ni in seawater by using external aqueous calibrations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.microc.2009.07.011.

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Las aguas con un alto contenido en sales disueltas son medios en los que los métodos clásicos de análisis para determinar la concentración de metales traza son difíciles de aplicar. Las altas concentraciones de metales alcalinos (Na^{+}, K^{+}), alcalinotérreos (Mg^{2+}, Ca^{2+}) y halogenuros (Cl^{-}), junto con los niveles extremadamente bajos de los metales traza, hacen difícil o imposible la determinación directa de estos elementos en el agua de mar por la mayoría de instrumentos analíticos convencionales.

Durante el presente trabajo de investigación se han desarrollado distintos métodos de extracción en fase sólida para la preconcentración de metales traza en el agua de mar. Para ello han sido empleados cartuchos comerciales de sílice C18 y diversos polímeros de impronta iónica. Los métodos desarrollados han sido aplicados para determinar la concentración de metales traza en diversos materiales de referencia certificados y muestras de agua de mar. Por último, uno de los métodos diseñados ha sido empleado para la realización de un estudio de la concentración de metales traza en las aguas de la Ría de Arousa, antes y después del accidente del petrolero "Prestige".